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ERRATA

- Page 7, line 21, add "The larvae bore in the stems of cacao." ✓
- Page 199, lines 10 and 11, delete "or eleven" ✓
- Page 206, 2 lines from end, insert after "Evaniids" "(in the restricted sense of Townes (1949) and Crosskey (1951))" ✓
- Page 206, last line, for "They" read "The members of this family" ✓
- Page 207, line 2, insert "believed to be" before "parasites" ✓
- Page 349, last line, for "in. deep" read " $\frac{3}{8}$ in. deep" ✓
- Page 745, fig. 3, delete "number of males number of females" ✓
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SOME INJURIOUS CURCULIONIDAE (COL.) FROM NEW GUINEA.

By SIR GUY A. K. MARSHALL

Among a number of insects sent in for identification by Dr. J. J. H. Szent-Ivany, of the Department of Agriculture, New Guinea, were several species of weevils that are reported as causing serious injury to cacao and coffee plants. Four of these are described below, and the types have been deposited in the British Museum (Natural History).

Of the two species of *Oribius* Mshl. (1956) specimens were also found in an interesting collection of New Guinea weevils kindly submitted by Dr. J. L. Gressitt, of the Bishop Museum, Honolulu.

Subfamily OTIORHYNCHINAE.

***Paratactus lihirensis*, sp.n. (fig. 1)**

♂. Derm black with dull black scales and markings formed of dense overlapping whitish scales; prothorax without dorsal markings, apart from a few scales in the basal and apical angles; elytra with a large irregular subquadrate whitish patch at the base between striae 2 and 8, and another transverse band at the top of the declivity that runs outwards from stria 1 to interval 8 where it expands somewhat and merges loosely into a narrow marginal stripe that runs from the middle to the apex; the underside rather thinly clothed with small round white scales and short recumbent white setae, except in the middle of ventrites 1 and 2 where there are setae only.

Head with the frons sloping steeply to the rostrum, the shallow longitudinal wrinkles largely concealed by scales; the eyes very convex, highest behind the middle. *Rostrum* nearly as long as broad, widening from the base to the genae; the dorsal area much narrowed by the large scrobes and bearing a fine median carina, and with a low elevation at the top of the declivity. *Antennae* with the scape strongly compressed, squamose and with coarse subrecumbent setae; the funicle with joint 2 as long as the cylindrical basal joint. *Prothorax* as long as broad, rather strongly rounded laterally, widest at about the middle, much

narrower at the apex than at the truncate and carinate base; the dorsum gently convex longitudinally, highest at the middle, with separated round shallow punctures, each containing a very small granule bearing a very short broad recumbent seta, the interspaces with dense black scales concealing the shining punctate integument. *Elytra* almost cordiform, very acuminate behind, the subtruncate



Fig. 1.—*Paratactus lihirensis*, sp.n. ♂.

base narrowly carinate; the shallow striae with small separated punctures, each containing a very short dark recumbent seta, striae 1-3 not becoming deeper at the apex; the flat intervals much broader than the striae, apparently rugulose on account of the dense small convex black scales, which on the disk usually conceal the rows of minute distant granules, these being much more evident on the lateral intervals; the setae very minute and inconspicuous on the disk, but becoming much longer and paler on the declivity. *Legs* red-brown, the femora blackish, the white scales denser along the dorsal edge of the femora.

Length 6-7 mm.

LIHIR Is. (north of New Ireland): Lagakot Plantation, 5 ♂, vii-viii.1955 (J. J. H. Szent-Ivany).

The insects were found attacking the leaves of cacao.

The species closely resembles *Paratactus carbunculus* (Heller) (1908), which, so far as we know at present, is restricted to New Britain, where it was taken on cotton by E. Ballard, and on pumpkin leaves by J. L. Froggatt.

P. carbunculus ♂ differs as follows:—*Head* with the eyes rather less convex and highest at the middle. *Prothorax* densely covered with small granules and a little more narrowed in front. *Elytra* more or less suffused on the disk by bluish-grey scales; rather more produced at the apex, the intervals with rows of distinct

granules, which become much more prominent on the dorso-lateral margins. *Antennae* with the scape not compressed and less abruptly clavate at the apex.

Dr. Szent-Ivany found at the same time three females which were entirely grey and without any white patches; and he thought they might be females of *P. lhirensis*. This is quite possible; but more definite evidence is needed since in the case of *P. carbunculus* we have females closely resembling the males.

Oribius destructor, sp.n. (fig. 2)

♂ ♀. Derm shiny black with markings formed of small round whitish scales which are very liable to be abraded; prothorax with very sparse small scales (always present on the propleurae) and a fairly dense median stripe from the base to the middle; elytra with a stripe on interval 1 from the scutellum to the top of the declivity, a dense complete stripe covering the lateral margin inwards to stria 9, and a less dense complete stripe on intervals 5 and 6, which on the



Fig. 2.—*Oribius destructor*, sp.n. ♂.

declivity spreads broadly outwards to unite with the lateral stripe; the sternum with a loose patch of recumbent white setae and narrow scales at the sides of the metasternum only; the venter of the ♂ with rather dense long soft erect pale setae, which are shorter, sparser and stiffer in the ♀.

Head with the vertex smooth and almost impunctate, the frons with a few coarse punctures and a short deep median sulcus; eyes moderately convex.

Rostrum longer than broad (7:5), very gradually widening from the base to the genae; the median dorsal area nearly parallel-sided, coarsely punctate, with a low smooth median carina and with a slight elevation between the antennae. *Antennae* black or piceous, with the funicular joints elongate, 2 a little longer than 1. *Prothorax* of the ♂ globose, very strongly rounded laterally, widest at the middle, a little broader than long, the arcuate apical margin narrower than the base, which is widely but shallowly sinuate with a broad carinate margin; the dorsum very strongly convex in both directions, highest at the middle, with close (but not quite contiguous), small oval low granules, which become much flattened towards the sides and base, and are replaced on the pleurae by very small sparse convex granules, each dorsal granule with a short recumbent pale seta; prothorax of the ♀ similar but narrower, the dorsal granules not (or but slightly) flattened laterally, and the pleural granules larger. *Elytra* narrowly ovate in the ♂, broader and rather more acuminate apically in the ♀, but the apex not produced downwards, the shallow striae with rather large close punctures separated by low flattened granules (sometimes almost obliterated) which often tend to link up laterally with the granules in the adjoining striae; the intervals not, or but little, wider than the striae with a sparse row of small low granules, each bearing a short suberect white seta. *Legs* black, with sparse erect white setae, the tibiae of the ♂ (especially the front pair) with a fringe of long soft erect setae, the front tibiae feebly denticulate beneath in both sexes, and those of the ♂ incurved at the apex; the hind femora of the ♂ reaching or slightly exceeding the apex of the elytra.

Length 5.0-6.5 mm.

NEW GUINEA: Eastern Highlands, 5,200 ft., 6 ♂, 11 ♀, x.1954, iii.1955 (*J. J. H. Szent-Ivany*—type); E. Highlands, Asaro Valley, 5,400 ft., 1 ♂, 2 ♀, x-xi.1954 (*McFarland & Lane*); Mt. Otto, 7,300 ft., above Kabebe, 4 ♂, 1 ♀, vi.1955; Goroka, 5,200 ft., 5 ♂, 3 ♀, vi.1955; Ahl Valley, Nondugl, 5,800 ft., 9 ♂, 2 ♀, vii.1955; Upper Jimmi Valley, Tsenga, 4,000 ft., 2 ♂, vii.1955 (all *J. L. Gressitt*); Western Highlands, Mt. Hagen, 1 ♂, x.1954 (*Szent-Ivany*), Tremearne, 5,500 ft., 1 ♂ (*D. J. Kingston*).

Dr. Szent-Ivany reports that this species is a serious pest of *Coffea arabica* in New Guinea, causing considerable shot-hole damage to the foliage and also feeding on the coffee cherries.

The adults were likewise found feeding on the following plants in the Asaro Valley (4,900-6,000 ft.), Eastern Highlands:—*Acalypha*, *Albizzia stipulata*, *Brassica oleracea*, *Citrus* spp., *Crotalaria anagyroides*, *Euphorbia pulcherrima*, *Ipomoea batatas*, *Jacaranda*, *Lagerstroemia indica*, *Morus* sp. and *Passiflora* sp.

Larvae were found round the roots of various weeds (Compositae, Gramineae).

***Oribius hostis*, sp.n. (fig. 3)**

♂ ♀. Derm rather dull black, prothorax without markings, but sometimes with sparse minute pale scales towards the base and on the pleurae; elytra with an ill-defined stripe of small round non-contiguous pale scales on interval 5 from the base to near the apex, without any sutural stripe but sometimes with an indefinite spot on the suture at the top of the declivity which occasionally expands laterally to join the discal stripe on each side; the lateral margin with an abbreviated indefinite pale stripe at about the middle or behind it; the underside with a patch of dense white scales at the sides of the meso- and metasterna.

Head with the vertex opaque, finely rugulose, the frons coarsely punctate, with a short median sulcus; the eyes gently convex. *Rostrum* as long as broad, gradually widening from the base to the genae; the median dorsal area coarsely punctate with a shallow abbreviated median sulcus, and no elevation at the top of the declivity. *Antennae* long and slender, red-brown, joint 2 of the funicle

very slightly longer than 1. *Prothorax* of the ♂ strongly rounded laterally, widest at or rather beyond the middle, slightly broader than long, the feebly arcuate apical margin narrower than the base, which is truncate with a narrow carinate margin; the dorsum moderately convex longitudinally, with dense small rounded granules that are not at all flattened towards the sides and base, but



Fig. 3.—*Oribius hostis*, sp.n. ♂.

become much sparser and smaller on the pleurae, the dorsal granules each with a very short recumbent white seta; prothorax of the ♀ somewhat narrower and less rounded laterally. *Elytra* as described for *O. destructor*, sp.n., but less acuminate apically in both sexes, with the granules on the intervals smaller and less conspicuous, and without any white sutural stripe; the apex produced downwards like a beak in the ♀. *Legs* red-brown, with sparse subrecumbent white setae; the tibiae of the ♂ without long fringes; the front tibiae distinctly denticulate in both sexes, and those of the ♂ not incurved at the apex; the hind femora very slightly exceeding the apex of the elytra.

Length 5–6 mm.

NEW GUINEA: Western Highlands, Wabag (4,900–6,700 ft), 5 ♂, 7 ♀, x-xi.1954 (*J. J. H. Szent-Ivany*); W. Highlands, Minj, 1 ♂, 1 ♀ (in cop.), xi.1954 (*R. S. Carne*—type); Eastern Highlands, Asaro Valley, Miramar, 6,000 ft., 1 ♂, vi.1955; Ahl Valley, Nondugl, 5,800 ft., 2 ♂, 2 ♀, vii.1955; Upper Jimmi Valley, Korop, 4,300 ft., 1 ♂, vii.1955; U. Jimmi Valley, Wum, 2 ♂, 3 ♀, vii.1955 (all *J. L. Gressitt*).

Though closely related to *O. destructor*, sp.n., this species presents many differences: it lacks the white sutural stripe on the elytra and the median stripe on the pronotum; the rostrum has a shallow sulcus instead of a median carina; the prothorax is much less globose and bears smaller and denser granules; the

tibiae lack the long fringes, and the sternum has two patches of dense white scales on each side.

Like *O. destructor*, this species is stated to be very injurious to the foliage of coffee.

Subfamily PACHYRHYNCHINAE.

Pantorhytes szentivanyi, sp.n. (fig. 4)

♂ ♀. Derm black, except the elytra which are red with all the margins (but not the suture) indefinitely blackish; the head and prothorax sparsely and unevenly covered with rather large round or broadly ovate, pale yellowish scales; the elytra each with five complete rows of small distinct spots formed of a few smaller narrower yellowish scales nearly always abraded on the disk; the underside with some scales at the sides of the sternum and ventrite 1.



Fig. 4.—*Pantorhytes szentivanyi*, sp.n. ♀.

Head with a few coarse punctures and a short median stria, and obtusely raised laterally above the eyes. *Rostrum* as long as broad (without the projecting jaws), parallel-sided; the dorsum on the basal half with a broad longitudinal furrow on each side and a large deep oval median depression containing a deep longitudinal sulcus; the anterior part flat, with a finely shallowly punctate median area and coarsely punctate laterally. *Prothorax* as long as broad, moderately rounded laterally, widest at or a little behind the middle, with a broad doubled basal carina; the dorsum moderately convex longitudinally, highest at or beyond the middle, with a large shallow transverse depression at the base, the dorsal surface smooth and shiny, with sparse small punctures, each containing a recumbent white seta; the rather large round yellowish scales unevenly scattered, and

on each side at about the middle a small rounded spot of dense scales lying in a very shallow depression. *Elytra* very rotund in both sexes, but somewhat broader and more acuminate behind in the ♀, the base strongly carinate except near the suture; the striae in the typical form almost entirely obliterated by numerous flattened granules, which occur not only on the intervals but also between the punctures in the striae; in unbraded specimens there are rows of widely separated small spots of scales on intervals 1, 3, 5, 7, 9, with rather larger flattened granules between the spots; in some examples the striae are clearly recognisable towards the base, but there are then small sparse granules on the intervals and in the striae. *Legs* black, with scattered short white recumbent setae, and also sparse scales on the femora only; the femora coarsely wrinkled transversely on the apical half; the hind tibiae flattened on the inner face and quite smooth on both sides in the ♂, but rugulose on the inner side in the ♀.

Length 10–14 mm.

PAPUA: Popondetta distr., 11 ♂, 9 ♀, xii.1955 (J. J. H. Szent-Ivany).

Very closely allied to *P. proximus* Faust (1899), which differs in having no granules on the intervals or in the striae on the elytra, and the scales when present are pale bluish or greenish; on the pronotum the punctures are rather more numerous, and the scales are much smaller and bluish; the apical half of the rostrum bears strong separated punctures throughout; and the femora have no scales. *The larvae bore in the stems of cacao.*

A LABORATORY STUDY OF THE CIGARETTE BEETLE, *LASIODERMA SERRICORNE* (F.) (COL., ANOBIIDAE) WITH A CRITICAL REVIEW OF THE LITERATURE ON ITS BIOLOGY.

By R. W. HOWE

(PLATES I & II.)

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The original work in this paper consists of a study of the life-cycle of *Lasioderma serricorne* (F.) under controlled conditions. The species has not been studied in the warehouse in Britain, for here it can be an important pest only in heated premises. Since well over two hundred papers deal with this species, it seemed worth incorporating in this paper a review of the published biological information. The papers dealing entirely with chemical control measures, and those dealing with aspects of the tobacco industry, have been omitted. Records of the species on particular foodstuffs have been included only in so far as they establish the liability of the product to attack. The most informative published works include an excellent monograph by Runner (1919), a very useful account of the species as a tobacco pest in Greece by Stamatinis (1935), a paper by Powell (1931), who bred the insect under constant conditions, and a number of valuable papers of a general nature produced by the United States Department of Agriculture and by the East Indies tobacco research stations.

Records of Foodstuffs attacked.

Although well over half of the papers on *L. serricorne* concern the infestation of tobacco, this species has probably the most varied taste in food of all storage insects. Many records of storage insects attacking produce are based only upon the discovery of a few adults on that kind of produce, but it is certain that *L. serricorne* can and does breed on a wide variety of commodities. In the literature there are many records of infestation of plant materials which include aniseed (Strong, 1920; Wilson, 1931), areca nuts (Harris, 1939), atta (Davis, 1947), bamboo (Strong, 1922), beans (Lever, 1944; Wilson, 1931), biscuits (Bodkin, 1919),

cassava (Montgomery & Bragdon, 1919), chickpeas (Back & Cotton, 1924), cocoa beans (Hayward, 1954; Nicol, 1941; Passmore, 1932), coffee beans (Anon., 1929), copra (Essig, 1921; Herford, 1939), coriander (den Doop, 1917; Lever, 1941; Wilson, 1931), cotton seed both before (Wille, 1940) and after harvest (Wille, 1934; Smee, 1930), cottonseed meal (Back, 1939b; Bissell, 1935), cumin (den Doop, 1917; Morris, 1938), dates (Miller, 1922), dried banana (Gowdey, 1927), dried cabbage (Lever, 1944), dried carrot (Linsley, 1944), dried fruit (Zeck, 1943), drugs (Madel, 1939), flax tow (Back & Cotton, 1930), flour (Fraenkel & Blewett, 1943a), ginger (Hargreaves, 1926; Jepson, 1935; Richards & Herford, 1930; Sheppard, 1926), grain (Back & Cotton, 1940; Bodkin, 1919), groundnuts (Risbec, 1941), herbs (Strong, 1920), and herbarium specimens (Mossop, 1950), insecticides containing pyrethrum (Lever, 1945), juniper seed (Smee, 1923), liquorice root (Maskew, 1920), nutmeg (Maskew, 1919; Richards & Herford, 1930), raisins (Jones, 1913), rhubarb (Schwarz, 1883), rice (Appert, 1953), seeds of trees (Ehrhorn, 1923) and of other plants (Saraiva, 1939; Wilson, 1931), spices (Fletcher & Ghosh, 1920; Maskew, 1919), yeast (Runner, 1919) and, of course, tobacco.

L. serricorne also breeds in animal matter such as dried insects and dried fish (Runner, 1919) and fishmeal and meatmeal (Fraenkel & Blewett, 1943a), and has been recorded attacking leather (Bodkin, 1919) and the stored wax of *Cocos coronata* (Bondar, 1942).

Damage to cloth, upholstery (MacNay, 1953), paper and books (Bodkin, 1919) has been recorded, and has generally been considered as incidental to the attack on the furniture stuffing (Back & Cotton, 1930) or on the book-binders' paste (Kalshoven, 1938; Corbett, 1931).

The present work was undertaken because *L. serricorne* has become the principal pest of stored cocoa in West Africa (Cotterell, 1952; Hayward, 1954). Almost all the previous biological information on this species has been derived from work using tobacco, which is a comparatively poor food. Here, wheatfeed, which is probably one of the most satisfactory foods, was used in most experiments. In Nigeria, Mr. J. Riley of the West African Stored Products Research Unit has been working on this species using foods of local importance such as cocoa beans and cowpeas, as well as studying the species in warehouses. Shepard (1943) states that maize meal plus yeast powder is the best food known.

Distribution.

Nowadays, *L. serricorne* is found throughout the tropical and subtropical parts of the world. Its range is restricted by low temperature and low humidity. Runner (1919) suggested that it was a native of the warmer parts of America, but its discovery in dried resin in the tomb of Tutankhamen (Alferi, 1931) suggests that it was present in Egypt about 1,500 B.C. The first record of its association with tobacco was made in Paris in 1848 (Runner, 1919). Although it was thought to have been imported from the U.S.A., the earliest record from tobacco there is 1886 (Tenhet & Bare, 1951). *L. serricorne* can live and fly out-of-doors in the tropics, where it has been recorded as living on fallen damaged cotton bolls in Peru (Wille, 1931) and Nyasaland (Smee, 1930). It is carried from one tropical country to another by trade, especially in tobacco, and is also carried regularly to cooler, temperate countries where its continuance depends on its entry into warm buildings. As long ago as 1889, Fauvel remarked on its importation into France in cigars and groundnuts of unstated origin. Elsewhere in the same paper, the groundnuts mentioned were stated to be imported from Senegal.

Morphological Differences between *Lasioderma* and *Stegobium*.

Porta (1929, pp. 438-439) gives a key to a number of species of *Lasioderma*. Of these species only *L. serricorne* has been found in stored produce. At least

nine other Anobiids, however, have been recorded in storage premises, *Catorama tabaci* Guér. on tobacco by Runner (1919), *C. herbarium* Gorb. on nutmegs by Richards & Herford (1930), *Necobium castaneum* (Ol.) (del Cid, 1934), *Neogastrellus librinocens* Fisher (Baek, 1939a), *Gastrallus indicus* Rtt., *G. laticollis* (Pic) and *Ptilinus testaceus* Pic (Kalshoven, 1938), all on books, *Anobium* spp. (Fletcher & Ghosh, 1920) on spice, and *Stegobium paniceum* (L.), which is extremely common on a wide range of products. There seems to be no real reason why the two common species, *L. serricorne* and *S. paniceum*, should be confused, but this has occurred. In one entomological paper referring to bees' nests (Rayment, 1935), *S. paniceum* has been figured over the name *L. serricorne*, and Kalshoven (1938) appears to doubt some of the records of *S. paniceum* attacking books in south-east Asia. Thus it is worth while recording the differences between these two species.

The antennae of the adult beetles are very distinct (Pl. I, fig. 1). In *L. serricorne* they are serrate whereas *S. paniceum* has the typical Anobiid antennae with the last three joints very long and broad. The antennae are often missing or difficult to see in dead specimens collected from warehouses. These beetles can be distinguished by the elytra which are striate in *S. paniceum* but not in *L. serricorne*.

A final confirmation can be made by mounting and examining the genitalia which are very distinct in both sexes. In the female *L. serricorne* the apodeme (Pl. I, fig. 2) is distinctly V-shaped whereas in *S. paniceum* this structure is Y-shaped with the arms of the Y very short. The tip of the ovipositor of *L. serricorne* is lightly chitinated (Pl. II, fig. 1, a). In *S. paniceum* (Pl. II, fig. 1, b) the tip of the ovipositor is heavily chitinated and is asymmetric, the right lobe usually being broader, always more heavily chitinated, and lacking the minute terminal joint. The bursa of *S. paniceum* bears no armature, but that of *L. serricorne* (Pl. II, fig. 2) carries about half a dozen round chitinous plates each armed with a tooth and also contains two chitinous rings. In the male, the armature of the aedeagus (Pl. II, fig. 3) consists of large spines in *L. serricorne*. In *S. paniceum* the aedeagus is heavily chitinated but appears to be unarmed.

The larvae can also be distinguished fairly easily, *L. serricorne* having somewhat longer hairs than *S. paniceum*. The head of the larva of *L. serricorne* is evenly rounded dorsally whereas that of *S. paniceum* is somewhat pointed (van Emden, 1925). The head of *S. paniceum* is weakly chitinated behind the anterior margin of the frons, but that of *L. serricorne* is strongly chitinated. The head coloration is distinct (figs. 1 & 2 of van Emden, 1925), for in *L. serricorne* a dark mark with an upwardly convex boundary extends halfway up the frons, marking off a clypeo-frontal area, whilst in *S. paniceum* the dark colouring ends in a straight line across the frons just above the mouth-parts. The bands of spicules or asperities across the tergites of the abdomen, which are typical of Anobiids, are obvious in *S. paniceum*, but small and lightly chitinated in *L. serricorne*. Also *L. serricorne* has an arolium (Böving, 1927) on each tarsus extending beyond the middle of the claws, which as a result do not appear to be terminal in a living larva. In *S. paniceum*, the arolium is absent. The spiracles of *L. serricorne* are circular; those of *S. paniceum*, as in the Ptinids (Manton, 1945), bear chitinous lips about as long as the width of the spiracles. In many respects, *L. serricorne* is more difficult to distinguish as a larva from the Ptinids, especially as it possesses a small anal sclerite. The chief difference is the position of the first thoracic spiracle (Böving, 1927; Manton, 1945), which in Ptinids is located laterally close to the anterior margin of the prothorax, but in *L. serricorne* is in the posterior half of the prothorax. The mandible in *L. serricorne* has two apical teeth as against one in Ptinids and the anal cushion which is asymmetrical in Ptinids is symmetrical in *L. serricorne*. Böving (1954) gives useful long descriptions of the larval characters of *S. paniceum* (pp. 109-110) and *L. serricorne* (pp. 153-154), particularly those of the head capsule.

The Developmental Cycle.

Experimental methods.

Several authors have investigated the life-cycle of *L. serricornis* in their local conditions, generally using tobacco as food. As a rule, the prevailing relative humidity is not mentioned. Sometimes only the month of observation is given without stating the temperature experienced. The only previous study of the life-cycle made over a range of constant temperatures and humidities was by Powell (1931) who used yeast as the food material. In the present investigation a good foodstuff, wheatfeed, was used over a wider range of temperature and humidity combinations and a number of other kinds of produce was used under one of the more favourable environmental conditions. When it was to be used as an oviposition medium, the wheatfeed was ground and passed through a sieve of 60 meshes to the inch. This food was conditioned to the requisite experimental humidities by placing it as a thin layer in petri dishes in desiccators containing a solution giving the desired relative humidity, except that food to be used at 80 per cent. R.H. or higher was conditioned at 70 per cent. R.H. to restrict the growth of mould as far as possible.

Eggs were usually obtained at the experimental temperatures at 70 per cent. R.H. by placing about 50 young adult beetles on a thin layer of fine wheatfeed in a 1-lb. jam jar for 24 hours. The beetles for egg-laying, which were obtained from stock cultures from an insectary maintained at 25°C., were used at about a week old since this species lays most of its eggs soon after emergence and is short-lived as an adult. Eggs were sifted from the food, using a sieve of 60 meshes to the inch. Since few, if any, eggs were obtained outside the range 25° to 35°C., eggs for use at temperatures outside this range were obtained at 30°C.

The eggs were incubated under the various experimental conditions, being kept in groups of ten in 2 × 1-in. glass tubes which were examined at the same time every day. On hatching, each larva was placed in a 2 × ½-in. glass tube containing about 220 mg. of conditioned wheatfeed measured out by eye, using a special bakelite spoon. Thirty larvae were usually set up at each combination of temperature and humidity and left undisturbed, except for the daily opening of the desiccator, until large larvae could be seen readily.

Thereafter, each tube was carefully examined daily at the same time with as little disturbance as possible, and the dates of cocoon construction, pupation, the appearance of the adult and the emergence of the adult from the cocoon were noted. Usually, cocoons were built with the glass tube forming part of the wall. When a complete cocoon was constructed away from the wall, only adult emergence could be recorded for that individual. Beetles were sexed as pupae. On emergence they were weighed to the nearest tenth of a milligramme and the sex checked by gently squeezing the abdomen, causing the genitalia to protrude.

The temperatures used ranged from 15° to 40°C. by steps of 2.5°C. Temperatures of 17.5°, 20°, 22.5°, 25° and 30°C. were obtained in constant temperature rooms; 15°C. was obtained in a constant temperature box built into a refrigerator, and all other temperatures in incubators. At most temperatures, relative humidities from 20 per cent. to 100 per cent. by steps of 10 per cent. were used, and at 30°C. an experiment was also introduced at 25 per cent. R.H. All humidities up to and including 80 per cent. R.H. were obtained by using solutions of caustic potash of the appropriate specific gravity (Solomon, 1951), but 90 per cent. was obtained by using a saturated solution of barium chloride and 100 per cent. by using distilled water.

The incubation period of the egg.

The effects of temperature and humidity on the hatching of eggs are summarised in Table I. No eggs hatched at 15°, 17.5° or 40°C. Some eggs hatched at temperatures ranging from 20° to 37.5°C., and at all these temperatures low

humidity inhibited hatching. Eggs hatched at 20 per cent. R.H. only at 30°C. and at this temperature there was very good hatching at 25 per cent. R.H. Below 30°C., down to 22.5°C. some eggs (at least 40%) hatched at 30 per cent. R.H. and a high proportion (over 75%) hatched at 40 per cent. R.H., but at 20°C. the effects of low humidity were a little more rigorous (Table I). At temperatures

TABLE I.

The maximum relative humidities, at various temperatures, at which less than 50 per cent. of eggs or none at all hatched, and the incubation period.

Temperature (°C.)	Maximum R.H. % for		Incubation period (days)	
	No hatching	Under 50% hatched	Higher R.H.	Lower R.H.
17.5	—	—	no hatching	
20	40	50	20.4-22.1	—
22.5	20	30	12.4-13.1	{ 14.9 at 40% 16.5 at 30%
25	20	30	9.4-10.5	13.9 at 30%
27.5	30	30	8.1-8.9	—
30	hatched 20	20	6.0-6.3	{ 7.3 at 30% 7.8 at 25% 8.8 at 20%
32.5	30	50*	6.3 at 50%	7.4 at 40%
35	20	50	5.3-6.2	{ 6.7 at 40% 7.2 at 30%
37.5	70†	90*	7.3 at 90%	8.1 at 50%
40	—	—	no hatching	

* Max. R.H. used at these temperatures.

† A few hatched at 50% R.H.

above 30°C., the unfavourable effects of low humidity were more marked and in no experiment was there a 50 per cent. hatch at 50 per cent. R.H. or less. At 32°C., Powell (1931) recorded a 70 per cent. hatch at 45 and 60 per cent. R.H. and over 90 per cent. hatch at 75 and 90 per cent. R.H.

Humidity does not appear to have any marked effect on the duration of the egg period except at the lower limits of humidity tolerance. In the favourable humidity region, the differences between the means of the egg periods are too small to be detectable by daily inspections for hatching. At the extremes of low humidity, however, the egg period is noticeably increased (Table I) and although statistical tests would not be valid, it is probable that this increase is real. It is probably due to delayed emergence rather than to slower development of the embryo. At 32°C., Powell (1931) found that at 45 per cent. R.H. the period was 9.7 days as against 5.5-6.7 days at higher humidities. The optimum temperature for the rapid development of the embryo is about 35°C. The results given in Table I correspond reasonably well, with slight exceptions, to the approximate data available in the literature for the variable conditions experienced in warehouses. These are summarised in Table II with the relevant details arranged

in ascending order of incubation period. From this Table it could be concluded that eggs develop and hatch at temperatures between 20° and 34°C. The value given by Powell for 20°C. agrees with the results of Table I. Bovingdon's (1931) figure of 8 days for 21°C. probably refers to variable conditions with a minimum of 21°C. The value given by Bare, Tenhet & Brubaker (1947) for 26.5°C. is smaller than that estimated from Table I.

TABLE II.

The incubation period of *L. serricorne* according to various published accounts.

Period (days)		Temp. (°C.)		Place	Conditions	Authority
Mean	Range	Mean	Range			
—	5-6	—	—	Caucasus	—	U
5.5	5-7	32	—	—	90% R.H.	P
5.8	4-8	32	—	—	75% R.H.	P
—	5-7	—	—	Caucasus	in factory	Sk
6	—	—	26-30	—	—	Sk
6.1	4-10	—	—	Philippines	May-Nov.	J
6	4-14	—	—	Java	—	V
6.2	6-7	32	—	—	60% R.H.	P
—	6-7	27	—	Germany	—	Mad, Z
—	6-8	30	—	—	80% R.H.	R
7	—	—	21-25	—	—	Sk
—	5-9	—	19.5-28.5	Greece	June-Oct.	St
7	—	—	—	S. Rhodesia	—	Mos
7	—	—	—	U.S.A.	summer	TB
7	—	26.5	—	—	70% R.H.	B
7	5-10	—	27-34	Florida	Apr.-May	R
—	5-10	—	—	Algeria	May-Sept.	DL
—	5-10	—	—	Japan	—	SY
—	7-8	—	—	Nyasaland	—	Sm
7.6	—	—	—	Tennessee	July-Aug.	R
—	7-14	—	—	Tennessee	May	R
8	—	21	—	—	in factory	Bov
—	6-10	—	—	U.S.A.	—	TB
8	6-10	—	—	Florida	July-Aug.	R
8.2	6-14	—	—	Virginia	July	R
—	8-10	—	18-30	Italy	Apr.-Aug.	C
9.7	7-11	32	—	—	45% R.H.	P
10	—	—	—	Bulgaria	spring	Mok
10	—	—	—	Jamaica	—	G
11	—	—	—	Philippines	—	M
11	—	—	—	N. Carolina	June	A
—	11-13	—	—	Bengal	Jan.	Cot
14	—	20	—	Java	Dec.	VR
20	—	20	—	U.S.A.	—	P

Author abbreviations: A, Atkinson, 1886; B, Bare, Tenhet & Brubaker, 1947; Bov, Bovingdon, 1931; C, Canzanelli, 1935; Cot, Cotes, 1894; DL, Delassus & Lepigre, 1931; J, Jones, 1913; M, Mackie, 1911; Mad, Madel, 1938; Mok, Mokrzhetzki, 1925; Mos, Mossop, 1937; P, Powell, 1931; R, Runner, 1919; Sk, Skalov, 1931; Sm, Smee, 1923; St, Stamatinis, 1935; SY, Shibuya & Yamada, 1935; TB, Tenhet & Bare, 1951; V, van der Veen, 1940; VR, Veth & van Rijn, 1915; Z, Zacher, 1927.

The larva.

In the absence of any other food, the newly hatched larva may eat the egg shell. Runner (1919) observed that newly hatched larvae could live 5-10 days without food. Zacher (1927) and Madel (1938) also state 10 days but van der Veen (1940) puts the maximum survival at 2 days. This obviously depends on the

temperature and humidity of the environment, but in any event it is unlikely that the larvae will ever be entirely without food except in a laboratory experiment. Newly hatched larvae are negatively phototropic (Bovingdon, 1931) and are extremely active. They will enter very small holes in search of food and so can penetrate into packed finished cigarettes and similar goods (Runner, 1919). The older scarabeiform larvae are less active, but are still capable of considerable wandering and they remain negatively phototropic. The larvae will feed on a surface or will bore into produce such as cigars, cereal grains, cocoa beans or stored ginger root. They will penetrate deeply into a stack of produce such as cocoa beans, in which there is plenty of air space but remain peripheral in closely packed materials such as meals (Back, 1939b).

When fully grown, the larva stops feeding and builds a cell wherever it finds a convenient spot. It usually requires a firm foundation such as is afforded by the midrib of a tobacco leaf, the lining of a cigarette packet (Runner, 1919), or the sacking containing produce (Back, 1939b), or it may build the cell inside a cocoa bean or cowpea. The cells are flimsy and fragile, made of food and waste material cemented together by a secretion produced by the midgut (van Emden, 1929). If the cell is attached to a surface the cement may be applied to it as a thin layer or it may be omitted over this area. When a larva applied the cement to the glass of our experimental tubes, it prevented observation of pupation and later metamorphosis. Disturbance may cause a larva to desert a partly made cell and start to build a fresh one or may even cause it to pupate without finishing the cocoon.

Larval activity ceases when the temperature falls to between 19.5° and 15.5°C. and the larva becomes dormant at slightly lower temperatures (Runner, 1919). It can remain dormant for many months and may overwinter in this stage in climates that are cool but not too cold, as in Tennessee (Runner, 1919), Greece (Stamatinis, 1935), Japan (Shibuya & Yamada, 1935) and Italy (Canzanelli, 1935). In colder countries the larvae die during the winter (Mansbridge, 1936) if exposed to outdoor temperatures but they may survive when protected from the cold by buildings or produce. Powell (1931) states that at a constant temperature of 22°C. less than a half of the eggs and pupae survive and only a fifth of the larvae pupate. In the present work, the mortality observed at a constant temperature of 20°C. was not as high.

The rate of larval development on wheatfeed and groundnuts.

The duration of the larval stage of *L. serricorne* on wheatfeed is given in Table III. At 37.5°C., a temperature at which only very few eggs hatched, larvae failed to pupate at 90 per cent. R.H., but grew fairly quickly, though with a high mortality, at 50 and 70 per cent. R.H. The optimum temperature for rapid development is about 32.5°C., except at relative humidities below about 40 per cent. where growth is quickest at 30°C. The speed of development decreased down to 20°C., at which temperature the developmental period is about 70 to 80 days. Development of the larva was not completed at either 17.5° or 40°C. There also appears to be an optimum relative humidity for rapid development at about 70-80 per cent. Development is slower at higher humidities with heavy mortality at the limiting temperatures. This may be due to the growth of moulds but this seems unlikely because excessive growth of mould at these humidities was prevented by replacing the food about half-way through the larval cycle. In some experiments, a mould depressant, ortho-hydroxy-phenol was mixed with the food at a level of one per cent. by weight. It was used at 100 per cent. R.H. at 22.5°, 25°, 30° and 35°C. and also as a control at 70 per cent. R.H. and 30°C. The results, compared with those of experiments lacking the mould depressant, were very erratic, but the rate of development was always slowed down. Sometimes the slowing was considerable, sometimes slight. Mortality was also increased

markedly in some experiments. It was not easy to mix the mould depressant evenly with the food and it is possible that some larvae received a high dose of the chemical. These may have been poisoned and may have died or, at least, been adversely affected.

The minimum humidity at which complete larval development is possible on wheatfeed depends on temperature, falling from 50 per cent. R.H. at 20°C. to

TABLE III.

The duration of the larval period on wheatfeed, together with the percentage mortality at various combinations of temperature and humidity.

R.H. (%)	Temp. (°C.)	No. pupated	Mean period (days)	S.E.	% died	Temp. (°C.)	No. pupated	Mean period (days)	S.E.	% died
100	20	6	76.3	5.82	77	30	24	20.2	0.45	17
90		25	77.6	4.60	14		23	20.1	1.10	17
80		24	69.6	4.10	10		29	18.2	0.27	0
70		25	69.6	1.32	14		29	18.4	0.30	3
60		23	77.8	2.20	11		30	19.9	0.21	0
50		14	67.9	1.46	17		28	21.6	0.63	3
40							28	25.0	0.96	7
30							22	33.2	0.48	20
25							23	38.1	0.62	11
20							0	—	—	100
100	22.5	27	44.1	1.03	7	32.5	P min.	19		
90		19	41.4	0.73	13		P min.	*16		
80		26	39.3	0.62	3		29	15.7	0.12	0
70		26	37.6	0.27	10		P min.	20		
60		26	42.3	0.78	7		22	19.2	0.16	3
50		20	47.5	0.83	12		25	26.7	0.42	17
40		28	64.8	1.34	7		14	50.0	1.86	53
30		0	—	—	100					
100	25	19	31.7	0.57	30	35	13	21.2	0.99	50
90		27	28.1	0.37	0		13	25.6	0.95	50
80		26	28.1	0.87	3		21	18.6	0.23	21
70		29	27.9	0.42	0		26	20.9	0.40	0
60		30	30.2	0.42	0		25	18.1	0.17	13
50		29	31.9	0.27	12		6	22.8	2.72	7
40		27	42.0	0.42	33		1	34.0	—	67
30		0	—	—	100		1	101.0	—	83
90	27.5					37.5	0	—	—	100
70		21	22.4	0.48	19		12	25.8	1.51	55
50		23	25.5	0.32	20		7	30.8	1.53	60
40		26	32.2	0.53	13					
30		23	52.3	0.87	17					

P, Figures given by Powell (1931) for 32°C.

* 75 per cent.

25 per cent. R.H. at 30°C., and then rising again to 30 per cent. R.H. at 32.5° and 35°C. and to 50 per cent. R.H. at 37.5°C. At 30°C. with groundnuts as food, larvae can grow at 40 per cent. R.H., but not at 30 per cent. This increased susceptibility of larvae to low humidity when fed on groundnuts as compared with wheatfeed has also been noted in *Tribolium castaneum* (Hbst.).

The length of the developmental cycle increased with decreasing humidity, particularly near the lower humidity limit (Table III). A few humidities were compared at 30°C. using whole groundnuts as food. A mean larval period of 28.4

days was obtained at 70 per cent. R.H. and of 37.2 days at 50 per cent. R.H. At 40 per cent. R.H. all the larvae pupated inside the groundnuts, so no figure was obtained for the larval period.

All the results quoted above were obtained using specimens bred in the laboratory insectary. A few experiments were performed using a strain bred from Nigerian cowpeas but the results obtained with these beetles did not differ from those shown by the insectary strain.

Throughout this work with few exceptions, to avoid any density effects, conclusions have been drawn from experiments made with insects reared singly. A series of experiments was done, however, in parallel with a similar group carried out by Mr. J. Riley of the West African Stored Products Research Unit in Ibadan. In these, his technique of seeding cultures containing an excess of food in a 3 x 1-in. glass tube with twenty newly hatched larvae was used. The dates of pupation, of adult formation and emergence and the weight of the emerged adults were recorded as usual, and no deleterious effects of crowding were recorded (see Tables VI & XI). This contrasts with previous experience with *Ptinus tectus* Boield. (Howe & Burges, 1953).

In a number of the experiments there were one or two individual larvae which grew much more slowly than the rest of the larvae under the same experimental conditions, often needing two to three times as long to reach the pupal stage. These have been omitted from the results given in this paper, because such results could increase the mean considerably. This omission of genuine experimental results has only been adopted where the exceptional value is far removed from a compact group. Where the slowness is likely to be a sign of adverse temperature, humidity or food conditions, the result must, of course, be retained, for an increase in the variability of the developmental period is one of the signs of

TABLE IV.

Values omitted from Table III because of the undue length of the larval period, with wheatfeed as food, and other similar values.

Temp. (°C.)	R.H. (%)	No. of normal larvae	Range of period (days)	Length of prolonged developmental periods (days)
35	100+	13	18-32	63
35	80	21	17-20	53
35	70	26	18-34	49, 54, 71
30	100+	15	17-30	39, 43, 57, 103
30	90	23	17-32	42
30	70C	8	17-21	60
25	100+	19	29-37	112
25	80	26	27-30	56
22.5	100+	27	38-59	95
22.5	90	19	36-49	86
22.5	80	26	36-49	61, 156
22.5	60	26	38-52	86
22.5	50	20	43-68	80
30 & 40	70*	37	16-48	60

+, With mould depressant added.

C, Control in experiment using other foodstuffs.

* Exposed to 40°C. for 1 week (see Table XIX).

adverse conditions. Even so, the conditions from which some values have been omitted are still in the main unfavourable, consisting chiefly of high (35°C.) or low temperatures (22.5°C.) or of those high-humidity experiments to which a mould depressant was added. Details of the omitted values are shown in Table IV.

Larval development on other foodstuffs.

There is little information in the literature with which to compare these figures because few authors quote either temperature or humidity. Powell (1931) worked at five constant temperatures and eight constant humidities using dried yeast cake as food. He obtained adults at all temperatures except 40°C. and at the four relative humidities, 45, 60, 75 and 90 per cent., but he gives details of the larval developmental period only for 32°C., and then only gives the minimum for each humidity. These are included in Table III and agree quite well with results given in that Table, being mainly a little larger than the present figures for 32.5°C. Bare, Tenhet & Brubaker (1947) bred *L. serricorne* at 26.5°C. and 70 per cent. R.H., which they thought was optimal. Using a mixture of 40 parts maize meal and 3 parts dried bakers' or brewers' yeast they obtained a larval developmental period of 35 days. Powell (1931) and Canzanelli (1935) both place the optimum for rapid development at 32°C. and 75 per cent. R.H. Delassus & Lepigre (1931) put the optimum at 28°C. to 32°C. Runner (1919) worked at 30°C. with various kinds of tobacco and obtained mean larval periods ranging from 29 days on plug chewing tobacco to 42 on cigarettes. On tobacco seed he obtained larval periods of 29 to 30 days and on pressed yeast cake 27 to 30 days. In an uncontrolled room in Tennessee during the summer, three larvae fed on tobacco seed pupated in 25, 31 and 38 days. There are several accounts of the life-cycle on tobacco in uncontrolled conditions. The data on the length of the larval developmental period on this food are summarised in Table V. Shibuya & Yamada (1935) state that the larval period of *L. serricorne* on stored ginger in Japan is 60-70 days.

TABLE V.

Published information on the length of the larval stage on tobacco in uncontrolled conditions.

Range of larval period (days)	Place	Conditions	Authority
27*	Algeria	optimal	DL
31-39	Caucasus	in factory (22-34°C.)	Sk
35	U.S.A.	summer	TB
29-50	Greece	spring to autumn (20-28°C.)	St
30-50	U.S.A.	—	R, TB
30-60	Java	—	V
42-49	Bulgaria	—	Mok
39-61	Virginia	Aug.-Nov. (10-33°C.)	R
50	Philippines	—	J
49-56	Nyasaland	—	Sm
35-70	Britain	in factory	Bov
42-66	Florida	Apr.-June (26-35°C.)	R
45-71	Tennessee	in room, summer	R
63	Java	spring, 30°C.	VR
60-70	N. Carolina	July-Sept.	A
151-170	Philippines	—	M+
about 150	Tennessee	overwintering	R
about 250	Greece	overwintering	St

* To building of cocoon.

Abbreviations as Table II, with the addition of M+, Mackie, 1917.

In the present work, a few foods other than wheatfeed were used as experimental foods for *L. serricorne*. These were mostly West African products, such as cocoa, cowpeas and groundnuts, but peas and wholemeal flour were included. It was not possible to record the larval period for products like cowpeas, into which

the larvae penetrated to feed, because pupation could seldom be observed, but Table VI gives the larval period at 30°C. and 70 per cent. R.H. for those foods on which the pupation dates were recorded.

Fraenkel & Blewett (1943*a, b*) also reared *L. serricorne* on a variety of foods at constant conditions, 25°C. and 70 per cent. R.H. Their results were given in the form of diagrams from which Table VII has been constructed. They failed (1943*a*)

TABLE VI.

Duration of larval stage in days and larval mortality of *L. serricorne* on several foodstuffs at 30°C. and 70% R.H.

Product	No. pupating	Mean \pm	S.E.	% died
Reared singly				
Wheatfeed	87	18.6	0.39	13
Wholemeal flour	20	19.3	0.17	0
Cottonseed meal	28	19.8	0.35	3
Crushed cowpeas	64	20.2	0.27	13
Yeast powder	28	21.0	0.29	0
Coconut meal	28	23.5	0.35	7
Crushed locust beans	24	26.5	0.55	17
Decorticated groundnuts	11	28.4	1.00	47
Crushed cocoa beans	15	47.8	4.69	11
Whole locust beans	9	61.4	9.45	67
Reared in groups of 20				
Wholemeal flour	67	19.1	0.24	16
Crushed cowpeas	56	21.2	0.43	30

to grow it on two natural foods, dried milk and dried fruit, attributing the failure respectively to too low and too high a moisture content. They also failed to grow it on an artificial diet containing the hygroscopic sugar, fructose, in place of the usual glucose. In practice, *L. serricorne* can infest dried fruit, and a number of infestations in dwellings in Australia have been traced to domestic foodstuffs, including dried fruit, in the pantries (Anon., 1946). In the laboratory experiments it is probable that the young larvae were drowned in the sticky fruit juices. With dried milk, the extremely low vitamin B content was probably an additional and possibly a major factor in preventing growth. Fraenkel & Blewett (1943*a*) also showed that larvae of *L. serricorne* can attack undamaged wheat grains. Most first-stage larvae can penetrate into the germ and a few eat it out completely, but only a very low proportion of adults emerge (Table VII). Part-grown larvae do much more damage and most are able to grow into adults. Canzanelli (1935) placed newly hatched larvae on wheat grains and found they died in 7-15 days. Wille (1931) found that completely sound cotton seeds were safe from attack by *L. serricorne*. Undamaged cowpeas may be resistant to attack by freshly emerged larvae, but are vulnerable to the older larvae. The latter point was established by setting up two small cultures of *L. serricorne* at 30°C. and 70 per cent. R.H. In each a number of young adults was placed on twelve holed cowpeas and twelve undamaged cowpeas marked with a cross in red ink. In less than one month the holed cowpeas were almost destroyed and the whole ones severely damaged. Some difference of opinion has arisen between Mr. J. Riley of the West African Stored Products Research Unit and myself as to whether the first-instar larva can attack undamaged cowpeas, since in parallel experiments, performed with apparently similar techniques in identical conditions, we have obtained contradictory results.

In practice the point as to whether undamaged cowpeas are susceptible to attack by young larvae of *L. serricorne* is unimportant so long as the stored cowpea is subject to severe Bruchid attack. The results of these experiments are shown in Table XI.

TABLE VII.

Duration of larval stage and larval mortality of *L. serricorne* when reared in groups of ten on several foodstuffs at 25°C. and 70% R.H. (Fraenkel & Blewett, 1943a, b).

Product	Larval period (days)		% died	Fraenkel & Blewett, 1943 a or b, fig no.
	Median	Range		
Flour, 85% meal	33.5	32-37	5	a, 3
Flour, wholemeal	34	32-37	5	a, 3
Flour, wholemeal	35.5	35-37	10	b, 3
Yeast powder	35.5	34-37	20	a, 14
Artificial glucose diet ..	37	35-40	20	b, 3
Flour, N.S.R.	38	37-48	20	a, 3
Artificial carbohydrate-free	38	37-41	25	b, 3
Pea flour	38.5	35-51	10	a, 14
English wheat	—	34-58	15-85	a, Table 5
Flour, patent	44.5	39-62	40	a, 3
Meatmeal	47.5	39-53	45	a, 14
Fishmeal	51	47-55	50	a, 14
Manufactured cocoa ..	116	98-130	55	a, 14
Wholemeal flour, 70% R.H.	32	31-36	10	a, 10
" " 50% R.H.	38.5	36-43	30	a, 10
" " 40% R.H.	—	—	100	a, 10
" " & less				

Published data on accessory food factors.

Fraenkel & Blewett have shown that *L. serricorne* can develop on foods devoid of carbohydrate (1943a) and on foods deficient in certain vitamins (1943c). These facts probably account for its ability to live on animal foodstuffs and on relatively poor foods such as tobacco. Fraenkel and his co-workers have devised an artificial basic diet which has been changed slightly from time to time. A typical formula is glucose, 50 to 80 parts (starch is not entirely vitamin-free); casein, 20 to 50 parts; yeast, 5 parts; cholesterol, 1 part; McCollum's salt mixture no. 185, 1 or 2 parts; water, 15 parts. If this diet is made carbohydrate-free by replacing the glucose by casein the rate of development is slightly slowed down and mortality is very slightly increased (Table VII). Omitting the yeast resulted in total failure of the larvae but the sterol could be omitted because enough of this is supplied by the yeast (1943a). The sterol is present in the water-insoluble fraction of the yeast. and larval growth is poor when both this fraction and the sterol are left out of the diet (1943b). This poor growth is due in part to lack of biotin. Extraction of wholemeal flour with chloroform (1943d) to reduce the sterol content renders it a poor food. This extracted flour can be restored to adequacy by addition of any one of several sterols, cholesterol (from animals), sitosterol (from plants), ergosterol (from micro-organisms) and 7-dehydrosterol but not by calciferol. Pant & Fraenkel (1950) were able to replace the yeast in the artificial diet by a mixture of nine B vitamins. On this diet the larval plus pupal period at 27°C. and 70 per cent. R.H. ranged from 26 to 35 days with 10 per cent. mortality. Any one of the nine vitamins could be omitted and some larvae would still be able to complete their cycle. Omission of thiamin raised mortality to 60 per cent., and omission of biotin raised it to 50 per cent.; the larval-pupal developmental period increased to 36-43 days and 42-52 days, respectively. Omission of any one of the other seven vitamins, riboflavin, nicotinic acid,

pyridoxin, pantothenic acid, choline, inositol and pteroylglutamic (= folic) acid had only slightly adverse effects. Pant & Fraenkel (*op. cit.*) showed further that *L. serricorne* does *not* require a sterol for normal growth when fed on this diet, although as has been already mentioned, Fraenkel & Blewett (1943*d*) created a deficient diet by extracting flour with chloroform. Presumably then some other essential substance was also removed. Pant & Fraenkel were able to show that

TABLE VIII.

Developmental mortality and length of larval instars and other stages of
L. serricorne on wheatfeed at 30°C. and 70% R.H.

Instar					No.	Period (days)		No. died
						Mean	± S.E.	
Larva I	33	3.9	0.06	7
" II	30	3.7	0.10	0
" III	30	4.0	0.06	0
" IV	28	7.6	0.57	1
" Total	28	19.3	1.05	8
Egg hatched to cocoon built	..				29	16.6	1.38	7
Larva in cocoon	28	2.8	0.44	1
Pupa	28	3.8	0.17	0
Egg hatched to adult emerged	..				28	26.8	0.74	8
Wt. of ♂ (mg.)	16	1.63	0.04	
Wt. of ♀ (mg.)	11	2.19	0.08	

the sterol and most of the vitamins are to some extent manufactured by symbiotic intracellular yeasts present in gut mycetomes. These symbionts can be killed in the mature embryo by surface sterilisation of the egg with alcoholic chloramine just before hatching. The treated eggs hatch and the larvae can be reared to

TABLE IX.

The pupal period and the period spent by the adult in the cocoon,
at various temperatures.

Temp. (°C.)	Pupal period (days)		Adult in cocoon (days)	
	Range of mean at different R.H.	Average	Range of mean at different R.H.	Average
20	11.3-12.8	12.3	9.4-13.1	12.1
22.5	7.7- 8.6	8.1	7.3- 7.9	7.6
25	5.9- 7.2	6.4	5.4- 6.9	6.1
27.5	4.7- 5.0	4.8	4.5- 5.3	4.8
30	3.3- 5.0	4.0	3.3- 4.4	4.0
32.5	3.5- 4.0	3.7	3.2- 3.7	3.5
35	3.2- 4.0	3.6	3.5- 4.7	4.0
37.5	4.0- 4.1	4.1	4.5- 5.4	5.0

adults with little difference in results from normal insects on the complete artificial diet. When any one of the five vitamins, riboflavin, nicotinic acid, pyridoxine, pantothenic acid or choline was omitted there was a complete failure

of growth. If either inositol, biotin or thiamin was omitted the results were similar to those with normal larvae. The absence of pteroylglutamic acid permitted 25 per cent. of larvae to develop in a period some ten days longer than normal. Omission of the cholesterol from the artificial diet did not affect the rate of growth but reduced survival from 70 to 35 per cent. Fraenkel & Blewett (1943c), using a basic diet which contained wheat germ oil and the insoluble fraction of yeast in place of biotin and with *p*-aminobenzoic acid in place of folic acid in the vitamin mixture, obtained similar results except that sterilised larvae failed completely on a thiamin-free diet instead of growing poorly like the normal larvae.

Larval instars.

Canzanelli (1935) states that there are four larval instars, but Jones (1918) and van der Veen (1940) state that there may be from four to six. In this work the number of instars was counted only at 30°C. and 70 per cent. R.H., in which conditions every individual moulted four times. The first three instars were approximately of equal length and the last was about twice as long. Most of the larval mortality was recorded in the first instar (Table VIII). The larva becomes a prepupa soon after completing the cocoon and at 30°C. casts the larval skin

TABLE X.

The pupal period according to various published accounts, probably including, in most, the adult period spent in cocoon.

Pupal period (days)		Temp. (°C.)	Place	Conditions or time	Authority
Mean	Range				
3	—	30	Java	Feb.	VR
—	3-6	—	Japan	summer	SY
5	—	—	Sumatra	—	deB
6	—	22-34	Caucasus	—	Sk
6	5-?	—	Florida	May-June	R
7	—	—	Florida	July	R
—	5-10	21	Britain	in factory	Bov
—	6-9	—	Japan	spring	SY
7.8	6-12	—	Virginia	room	R
7.8	—	—	Tennessee	July-Aug.	R
8	—	26.5	—	—	B
8	7-?	—	U.S.A.	summer	TB
8	7-9	—	Virginia	Sept.	R
—	6-10	—	Virginia	summer	R
8.1	—	—	Virginia	July-Aug.	R
8.1	—	—	Florida	Oct.	R
—	6-13	—	Algeria	optimal	DL
—	6-13	19.5-28	Greece	May-Sept.	St
—	9-?	32	U.S.A.	—	P
—	10-11	—	Caucasus	Sept.	U
—	10-14	—	Nyasaland	—	Sm
—	10-14	—	Java	—	V
*12.5	11-14	—	Philippines	—	J
13.6	—	—	Tennessee	Apr.-May	R
14	—	—	Bulgaria	—	Mok
15	—	—	Philippines	—	M
—	11-20	18-30	Italy	Apr.-Aug.	C
—	14-18	—	U.S.A.	cooler weather	TB
20	18-23	18-22	Italy	Apr.-May	C
—	20-24	17-?	Italy	Sept.-Oct.	C

Abbreviations as Table II, with the addition of deB, de Bussy, 1917b.

* From building of cocoon to emergence of adult from it.

after 2 to 4 days in the cocoon. According to Runner (1919), the prepupal stage lasts 4 to 12 days; Bovingdon (1931) quotes 6 to 12 days and Bare, Tenhet & Brubaker (1947) state 5 days at 26.5°C. and 70 per cent. R.H. Runner states that this stage can withstand low temperatures (apparently about 15°C.) for a considerable time.

The pupal period.

Pupae transform into adults under all the conditions in which larvae can develop. Humidity has no discernible effect on the rate of development, which is quickest in the region of 32.5° to 35°C. The effect of temperature is shown in Table IX. Pupal mortality was slight throughout and did not exceed two individuals in any experiment. The lengths of the pupal period as stated by authors in published literature are given in Table X. It appears, as it did with the egg, that Bovingdon underestimated the average temperature of heated premises in Britain. There are a number of other high estimates for the length of this period. It is probable that most of the figures quoted include the quiescent period spent in the cocoon by the maturing adult. Thus the minimum figure of nine days given by Powell (1931) for 32°C. exceeds slightly the sum of the pupal and cocoon periods obtained in this work. Some of the estimates given in Table X may even be timed from the building of the cocoon rather than from pupation.

The adult in the cocoon.

The adult remains inside the pupal cocoon for a period during which it hardens off and also becomes sexually mature. Runner (1919) and de Bussy (1917b)

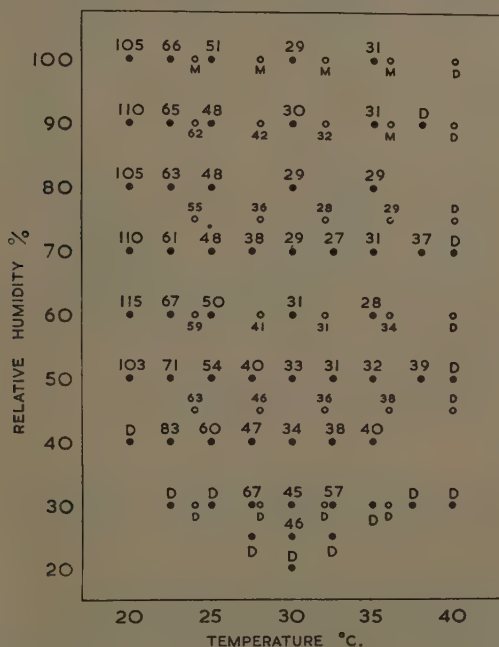


Fig. 1.—The period from hatching of the eggs to the first emergence of an adult from a cocoon when reared on wheatfeed (large figures) as determined in the present work, and on yeast (small figures) according to Powell (1931).

estimate that this period lasts five days and Jones (1913) records four days. The results of the present work are given in Table IX and show that the length of time the adult remains in the cocoon depends on temperature. It is not affected by humidity.

The length of the complete developmental cycle.

When a larva of *L. serricorne* bores into a foodstuff and the complete developmental cycle is passed inside it, hidden from view, the only period which can be measured is that from hatching (or from oviposition) to the emergence of the adult from the food. This period is also available for those foodstuffs in which every stage of the cycle is visible. In general, the egg and pupal stages are not affected by food, and usually the period the adult spends in the cocoon is unaffected by the food. The length of the complete cycle therefore, can be used fairly safely as a basis for comparing the nutritive value of foodstuffs when the larval period is not available. The data for the period from hatching to adult emergence from the cocoon are given in fig. 2 for wheatfeed. The limits for development and the regions of high mortality indicated by shading reflect these of the larval stage since nearly all the mortality observed was recorded in the first larval instar. Powell (1931) obtained no adults at any temperature at 30 per cent. R.H. and the speed of development he obtained on yeast was slightly slower than was obtained in this work on wheatfeed (fig. 1).

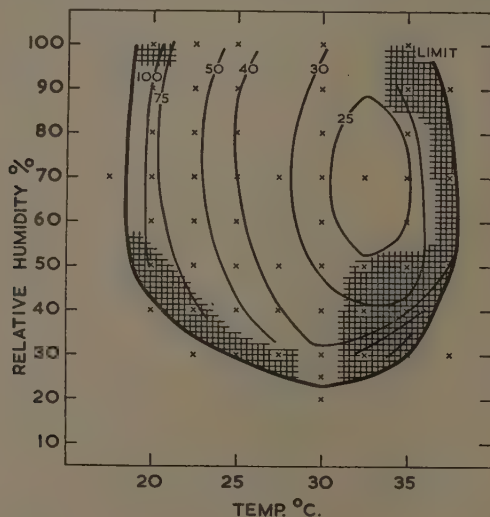


Fig. 2.—The complete developmental period and limits for development of *L. serricorne* when fed on wheatfeed. Shading shows conditions in which developmental mortality reaches 50 per cent.

The lengths of the developmental period measured from hatching on a number of foodstuffs at 30°C. and 70 per cent. R.H. are given in Table XI. With materials such as beans, the emergence of the adult may be betrayed only by the emergence hole if the beetle has returned inside the bean. Development was rapid on the cereal products, on cowpeas, yeast and on cottonseed and coconut meals. It was also relatively quick on whole groundnuts which are soft, but was slow on locust beans and cocoa beans. Cocoa is apparently a poor food, since

development was also slow on the crushed cocoa beans, but the slowness of development on locust beans is due to hardness of the beans, for the insect did quite well on the crushed locust beans. English garden peas were extremely resistant to attack by *L. serricorne* and only one beetle out of thirty emerged in 33 days. Some experiments were carried out at lower humidities with this food-stuff. Four beetles emerged at 30°C. and 50 per cent. R.H. in a mean of 41.25 days but none emerged at 40 per cent. R.H.

TABLE XI.

Developmental period from hatching to emergence of the adult from the cocoon and developmental mortality at 30°C. and 70% R.H. on various foodstuffs.

Product	No.	Period (days)			Mortality (%)
		Mean	±	S.E.	
Reared singly					
Wheatfeed	115	26.1		0.36	12
Wholemeal flour	21	26.5		0.27	0
Cottonseed meal	28	26.9		0.32	7
Crushed cowpeas	68	27.5		0.54	14
Damaged cowpeas	18	28.2		1.48	10
Yeast powder	28	28.4		0.27	0
Coconut meal	26	31.2		0.46	7
Whole cowpeas	25	31.6		1.64	37
Whole peas	1	33.0		—	97
Crushed locust beans	25	33.6		0.53	17
Whole groundnuts	30	34.4		5.26	50
Crushed cocoa beans	19	55.2		3.19	14
Whole locust beans	9	68.5		9.46	67
Whole cocoa beans	9	105.6		8.56	85
Reared in groups of 20					
Wholemeal flour	72	26.5		0.83	10
Crushed cowpeas	78	29.1		0.51	5
Damaged cowpeas	54	31.1		1.15	33
Whole cowpeas	56	32.3		1.27	30

An attempt was also made at 30°C. to determine the effect of humidity on the rate of development with groundnuts as food. Although mortality was high it was clear that decreasing humidity lengthened the complete developmental cycle (Table XII). The lower humidity limit for development is higher than it is with wheatfeed as food. Powell (1931) obtained the same lower humidity limit of 45 per cent. for both the foods he used, yeast and tobacco. These, like groundnuts.

TABLE XII.

The length of the complete developmental cycle (egg hatched to adult emerged) on whole decorticated groundnuts at 30°C. and at different relative humidities.

R.H. (%)	No.	Period (days)			Mortality (%)
		Mean	±	S.E.	
70	15	33.9		0.91	50
50	17	46.0		2.70	39
40	8	78.8		5.82	73
30	0	—		—	100

have a lower moisture content than wheatfeed at all humidities. Powell found that the total developmental period was 18 to 20 days longer on tobacco than on yeast under all conditions. De Bussy (1917b) showed development was slightly quicker on maize and rice than on tobacco.

Three West African products—cowpeas, cocoa beans and locust beans—were used as culture media to provide additional information on the value of these products as food for *L. serricornis*, 25 adults being added to each of two jars of each product at 70 per cent. R.H. at both 30° and 25°C. On cowpeas at 30°C. the insect multiplied some 30 times in three months and destroyed the cowpeas completely. On locust beans the increase was only eight times and feeding was restricted to broken beans and the mucous coat of whole beans. The bottoms of the jars were covered with dust. A later experiment showed that whole beans from which the mucous coat had been stripped could not be attacked. The cocoa beans used, although damaged before use, were attacked very little and the population had only doubled, one adult and one larva for each original adult, in the three months at 30°C. At 25°C. a similar pattern of results was obtained.

Powell (1931) found that grinding tobacco into fine particles before using it

TABLE XIII.

Length of the complete developmental cycle from egg-laying to emergence of adult from cocoon, mostly when breeding on tobacco, according to various authorities.

Period (days)		Temp. (°C.)	R.H. (%)	Place, conditions and food, if not tobacco	Authority
Mean	Range				
—	30-35	32	50-80	dry yeast cake	Sh
44	—	30	—	Sumatra	deB
44	—	22-34	—	Caucasus	Sk
—	40-?	—	—	room, yeast cake	P
—	42-49	—	—	Java	V
—	46-?	32	75	yeast cake	P
47	—	—	—	U.S.A.	Ch
—	42-56	—	—	U.S.A.	BC
—	49-?	32	60	yeast cake	P
—	49-?	36	75	yeast cake	P
50	—	26.5	—	maize meal and yeast	B
—	42-63	—	—	Sumatra	deB
56	—	—	—	U.S.A., summer	TB
—	50-60	—	—	Mauritius, summer	Jep
—	45-70	—	—	Virginia, summer	R
—	55-?	32	45	yeast cake	P
—	55-?	28	75	yeast cake	P
—	46-69	15-28.5	—	Greece, June-Oct.	St
60	—	24	50-80	dry yeast cake	Sh
64	59-67	—	—	Virginia, June-Sept.	R
—	57-?	—	—	room, tobacco	P
—	68-88	—	—	Java, May-Aug.	K
—	70-?	24	75	yeast cake	P
—	70-90	—	—	Nyasaland	Sm
80	—	30	—	Java, Dec.-Feb.	VR
—	86-?	—	—	India, Jan.-Apr.	Cot
90	—	—	—	N. Carolina, June-Oct.	A
about 275	—	—	—	Greece, overwinter	St

Abbreviations: A, Atkinson, 1886; B, Bare, Tenhet & Brubaker, 1947; deB, de Bussy, 1917b; BC, Back & Cotton, 1940; Ch, Chittenden, 1896; Cot, Cotes, 1894; Jep, Jepson, 1939b; K, J. C. Koningsberger in VR; P, Powell, 1931; R, Runner, 1919; Sh, Shepard, 1943; Sk, Skalov, 1931; Sm, Smee, 1923; St, Stamatidis, 1935; TB, Tenhet & Bare, 1951; V, van der Veen, 1940; VR, Veth & van Rijn, 1915.

as a food for *L. serricorne* increased the length of the developmental cycle from 51 to 70 days. With *Ptinus tectus*, Howe (1949) observed the opposite effect, fine particles enabling quicker development. Powell noted a range of period from 50 to 74 days on different grades of tobacco.

Published results for the length of the complete life-cycle from egg-laying to adult emergence are given in Table XIII. The figures for constant temperature given by Shepard (1943) and by Bare, Tenhet & Brubaker (1947) are in good agreement with those obtained in the present work (fig. 7). On tobacco, Powell (1931) recorded a developmental mortality of approximately 50 per cent. at 32°C. and 45–75 per cent. R.H. as against 40 per cent. in dry conditions and 13 per cent. at the optimum humidity on yeast.

The weight of freshly emerged adults.

The weight of the beetles bred in an experiment may provide an additional means of evaluating the suitability for *L. serricorne* of different environmental conditions and foodstuffs. In a short-lived non-feeding adult, it is also probable that the number of eggs laid by the female is directly related to her weight at emergence. Accordingly, each individual from each life-history experiment was weighed on the day it emerged from the cocoon.

In every set of conditions the mean weight of the female was greater than that of the male. In both sexes, insects bred at the highest humidities tended to be the heaviest. The heaviest insects were obtained at 25°C. Higher breeding temperatures caused a marked decrease of weight and lower breeding temperatures a slight decrease in weight. The lightest insects were obtained under the very hot, very dry conditions.

The heaviest mean weight recorded for females was 3.78 mg. at 25°C. and 100 per cent. R.H. A mean of 3 mg. was exceeded only at 25° and 22.5°C. at relative humidities down to 50 and 60 per cent., respectively. The heaviest mean weight for males was 2.73 mg., also at 25°C. and 100 per cent. R.H. The lightest mean weight for females was 1.57 mg. at 32.5°C. and 30 per cent. R.H. The average female weight was under 2 mg. for the lowest humidity at which the species developed for every experimental temperature above 25°C. The lightest males were bred at 27.5°C. and 30 per cent. R.H. (1.31 mg.), 30°C. and 25 per cent. R.H. (1.32 mg.) and 37.5°C. and 30 per cent. R.H. (1.39 mg.).

Weights of adults bred on foods other than wheatfeed were generally slightly lower, but on the whole, for *L. serricorne*, weight is not a very sensitive means of comparing the value of different foods. Jones (1913) notes, however, that adults bred from high-grade tobacco are much larger than those bred from a low grade.

The Adult Beetle.

Habits of the adult beetle.

The adult of *L. serricorne* does not feed, but as Mackie (1917) points out it can perforate tobacco and bite its way out of its cocoon. Hence it is frequently reported as feeding. It will take fluids, and drinks readily if allowed access to moisture or damp cotton-wool. It lives in dimly lighted places, often in crevices, and Shepard (1943) recommends putting a rolled-up band of corrugated paper about 1 in. wide into cultures to provide a refuge for adults. Adults of *L. serricorne* avoid bright daylight but at night they are attracted to some types of artificial light. Presumably this is what is meant by Canzanelli (1935) when he states that adults are attracted to light. Reed, Morrill & Livingstone (1934) state that lamps of 100 watts or more repel, and that dimmer ones attract, adults of *L. serricorne*. Melia, Melia & Lepigre (1934) and Reed, Morrill & Livingstone (1935) found that a 50-watt bulb was the most attractive. Runner (1919) found the blue end of the spectrum most attractive. The adults are very active in dim light, especially at sunset, and their activity may continue throughout the night. They

are also extremely active if trapped in bright sunlight, a reaction to warmth together with an attempt to escape from the light. Delassus & Lepigre (1931) state that the beetles seek out crevices and also state that eggs are laid in dust in such sites. Adults are inactive at temperatures below 18°C. (Bovingdon, 1931; Stamatinis, 1935).

Length of life.

The adult is short-lived, the length of life depending mainly on temperature and humidity, being shortened by high temperature (Table XIV) and low humidity. At 32°C., Powell (1931) showed an increase in adult life from 12 days at 45 per cent. R.H. to 16 days at 90 per cent. Unfertilised females may live longer than other individuals. Powell (1931) found that virgin females lived as long as 2 to 3 months. No systematic attempt was made in this work to determine the length of adult life at constant conditions but some data for 70 per cent. R.H. accrued from the experiments on oviposition. These data are given in Table XIV together

TABLE XIV.

Mean length of adult life at various temperatures at 70 per cent. R.H. and also in uncontrolled conditions according to various authors.

Constant conditions			Variable conditions	
Temperature (°C.)	Length of life (days)		Author	Length of life
	♀	♂		
20	46	43	Back & Cotton (1940)	2-4 weeks
22.5	30	37	Bovingdon (1931)	3-6 weeks
25	31	28	Runner (1919)	3-6 weeks
30	24	26	Skalov (1931)	11-23 days
35	18	21	Ustinov (1932)	27-31 days
27.5	18	19	Canzanelli (1935)	20-45 days

with some of the published information on the length of life of adults. Runner (1919) found that, in Tennessee, females lived longer than males by 3 to 19 days, and Stamatinis (1935) records female life as 30.6 days against 21.2 days for males. The mean figures obtained here show no consistent difference between the sexes. In most pairs the male died first but a few males lived exceptionally long lives and raised the mean for that sex.

Flight habits.

In warm countries the flight of adults may be very important in the local spreading of the species to almost every infestible material in an area (Runner, 1919). Canzanelli (1935) appears to claim they are active in bright sunlight, as indeed they would be if disturbed in such conditions by the unpacking of material, and Ustinov (1932) states that mature beetles containing eggs are positively phototropic and fly to warehouse windows. Normally, however, they fly only during the late afternoon, at dusk and during darkness (from 5 p.m. to dark according to Reed, Morrill & Livingstone, 1934), and sometimes during daylight on warm dull days (Back, 1939b). Tenhet (1955) showed that in buildings in which the light was never bright, flight was very slight after 2 a.m. until 2 p.m. Over half the total catch in a light-trap was made between 6 and 10 in the evening. In countries where the life-cycle is controlled by the climate in such a way as to restrict the appearance of adults to definite seasons, as in Bulgaria, there are definite seasonal flights at these times, *i.e.*, May and August-September in

Bulgaria (Mokrzhetzki, 1925). Den Doop (1919) makes practical suggestions which imply that the flight range is not more than 0.75 mile.

Sex ratio.

In the present work no marked deviation from an even sex ratio was obtained. About 49 per cent. of the 1,300 individuals bred singly on wheatfeed were female, but about 51 per cent. of some 400 individuals bred on other foods were female. The most extreme female-male ratios obtained in any experiment producing over ten adults were 4:21 and 12:4. There were no marked deviations from a sex ratio of unity when experiments were grouped according to temperature or humidity. Runner (1919), on the other hand, found females predominating. In laboratory material he obtained 41 females (53%) to 36 males and while collecting at light he took 64 females in a sample of 100 beetles. These observations agree well if the shorter male life that he observed is taken into account. Powell (1931) had 54 per cent. females in laboratory stock. Reed, Morrill & Livingstone (1935) found 36.6 and 43.6 per cent. males among beetles caught by light-traps in two years.

Oviposition.

The preoviposition period of *L. serricorne* is given as 2-3 days at 26.5°C. and 70 per cent. R.H. by Bare, Tenhet & Brubaker (1947). Dick (1937) gives it as 6-8 days at 27°C. and 73 per cent. R.H., but it seems likely that he was working from the "emergence" of the adult from the pupa and not from the cocoon. If this is so, these observations agree well. In uncontrolled conditions, the preoviposition period has been recorded as 1-3 days (Stamatinis, 1935), 2 days (Canzanelli, 1935), 2-5 days (Jones, 1913), 2-6 days (Runner, 1919), 4-5 days (Ustinov, 1932) and few up to 10-13 days (Skalov, 1931). The length of the preoviposition period including the time spent in the cocoon depends upon temperature (Table XVI) but no effort has been made here to determine if it is affected by humidity. It seems probable that sexual maturation ensues during the period spent in the larval cocoon, for mating and oviposition commenced within three days of emergence from the cocoon at all temperatures at which these functions were possible. Virgin females were not observed to lay any eggs.

In the present work, all the pairs were obtained by sexing newly formed adults which were removed from their cocoons. The sexing was done by squeezing out the tips of the genitalia by gentle pressure. As there was some risk of damaging females by this method, all pairs not producing eggs were discarded. At each temperature, the preoviposition period exceeded, by two days at most, the period given in Table IX for the adult in the cocoon. Only two females laid at 20°C. which corresponds closely with the views of Runner (1919) and Zacher (1927) that oviposition does not usually occur below 21°C. A few infertile eggs were laid at 37.5°C. (Table XV). Powell found that increasing the density to 12 pairs in a 4-oz. jar did not affect the number of eggs.

Mating will take place several times. Eggs are laid singly in crevices, folds or depressions in the food and the number of eggs is depressed in the absence of food (Dick, 1937). Shepard (1943) considers the beetles need a hard surface on which to crawl and that they lay more readily on such a surface. Runner (1919) states that oviposition is stimulated by smell and Powell (1931) obtained a few eggs on filter paper soaked in tobacco extract. Powell also found that a medium with no food value such as sawdust or sand or a surface such as sacking also permitted oviposition but that no eggs were laid in empty jars. The temporary absence of an oviposition site reduces the number of eggs. In natural conditions, *L. serricorne* probably oviposits whenever the temperature is favourable in produce, dust, sacking and other suitable places. According to Jones (1913), the time of most active laying is the early part of the evening. Mossop (1937) records laying in

spring (September and October) in Southern Rhodesia, but as he states that there are at least two generations a year, presumably there is laying until the next cool season by any adults that emerge. Runner (1919) notes that in Virginia eggs are not laid in unheated buildings between November and April.

Dick (1937) states that oviposition continues for 6 to 9 days at 27°C. and Powell (1931) records 3 to 10 days at 32°C. In the present work, individual females laid eggs for 6 to 9 days at 35°C., 7 to 10 days at 30°C., 11 to 18 days at 25°C. and 14 to 20 days at 22.5°C. The longest oviposition periods recorded by Runner (1919) were 21 days in Tennessee and 11 days in Florida. Stamatinis (1935), working in the laboratory in Greece, recorded oviposition periods of 4 to 14 days in July, increasing to 8 to 19 days in September. Skalov (1931) had oviposition periods of 8 to 15 days in February to April in the Caucasus, and Jones (1913), 6 to 8 days in the Philippines.

For a species with a short-lived adult, the average number of eggs per female is a very useful figure. This has been given as 20 by Shibuya & Yamada (1935), 27 and 32 by Runner (1919), 30 by van der Veen (1940), 38.6 by Stamatinis (1935), 39-45 by Ustinov (1932), 43.6 by Powell (1931), 40-75 by Atkinson (1886), 69.3 by Skalov (1931) and 76.1 by Dick (1937). The range quoted by Powell for a hundred pairs was 1-95, by Stamatinis for twenty pairs it was 19-73, and by Dick for ten females it was 55-93. Skalov (1931), with fifteen pairs, obtained a range of 24-112. Runner obtained 103 eggs from a pair at 30°C. and 80 per cent. R.H. Back & Cotton (1940) put the maximum egg output at about 100. The highest figure obtained here was 172 at 30°C., with the maximum over 100 at all temperatures from 22.5°C. to 35°C. (Table XV).

TABLE XV.

Average egg output and percentage hatch at various temperatures at 70 per cent. R.H. on wheatfeed.

Temp. (°C.)	No. of laying pairs	Total eggs	Mean per ♀	S.E. of Mean	Min.	Max.	% hatch	
							Mean	Range
20	2	90	45	5.66	37	53	50	43-55
22.5	9	810*	91.4	3.56	38	140	78	61-89
25	4	426*	115.2	5.53	103	126	76	68-82
30	12	1190	99.2	9.79	28	172	66	16-92
30N	14	935	66.8	7.54	21	112	—	—
35	9	985*	116.8	6.26	82	145	60	45-70
37.5	2	20	10	1.00	9	11	0	—

N, Water not provided.

* A female lost or killed during experiment.

Riley (unpublished) has counted the egg-rudiments in females bred on a number of foodstuffs and obtained averages ranging from 18.9 for females reared on cocoa to 51.4 for those reared on wholemeal flour.

At 20°C., only two of the ten females laid. At 37.5°C., no fertile eggs were laid, although two females produced a few eggs. Provision of drinking water or damp filter paper improved egg production both in average and maximum at 30°C. and 70 per cent. R.H., the only condition in which oviposition was measured without supplying water.

Percentage of eggs hatching.

At 20°C., only 50 per cent. of the eggs laid by the pairs hatched at 70 per cent. R.H. (Table XV). At 22.5°C., 78 per cent. hatched, and with increasing

temperature the proportion hatching fell consistently to 60 per cent. at 35°C. The percentage hatch was also investigated for eggs laid by individuals taken from stock cultures, some of the larvae hatching being used in the life-history experiments described earlier in this paper. At 37.5°C., hatching was very poor, the highest percentage hatch being 16. At 30°C., between 60 and 80 per cent. hatched at all humidities down to 25 per cent. R.H., but at 20 per cent. this proportion fell to 44. At all other temperatures there was a similar sharp increase in egg mortality near the lower humidity limits which differ according to the temperature (Table I). In general, the percentage hatch varies between 60 and 90 at favourable humidities, being better the higher the humidity. Most published papers report better hatching than this. Runner often obtained the complete hatch of a batch of eggs, and records the hatching of 86 per cent. of 182 eggs at 29°C. and 96 per cent. of 773 eggs at room temperature. Jones (1913) estimates that just over 95 per cent. of eggs hatch, and Canzanelli (1935) gives the usual hatch as 87-97 per cent.

Oviposition pattern.

The oviposition pattern (Table XVI) is of the type in which laying reaches an early maximum and falls off rapidly. Dick (1937), working at 27°C. and 70 per cent. R.H., found that 35 per cent. of the eggs were laid on the first of the nine

TABLE XVI.

The average rate of oviposition of fertile females on wheatfeed at 70 per cent. R.H. and various temperatures.

Temperature (°C.)	20	22.5	25	30	30N	35
Shortest preoviposition period (days)	12	7	6	4	4	4
Day*						
1	6.0	5.4	3.0	19.8	26.9	13.2
2	6.5	12.4	11.0	18.8	16.1	28.3
3	7.5	8.3	13.3	11.4	8.0	17.9
4	4.0	7.9	12.0	11.8	5.5	16.1
5		10.0	9.3	8.3	5.2	11.8
6	7.5	6.8	11.5	5.4	2.5	11.2
7		6.1	7.5	9.1	1.2	11.0
8		6.2	8.3	5.7	1.0	5.3
9	1.5	6.2	9.4	4.1	0.4	1.8
10	5.0	3.1	6.3	4.8		0.3
11-12	6.0	8.7	11.7	0.1		
13-16	1.0	6.6	8.7			
16+		3.5	3.3			

N, No water supplied.

* Measured from day on which the first egg was laid.

days of laying. A similar result was obtained in the present work at 30°C. when no drinking water was provided. When water was supplied, the oviposition period was slightly prolonged and the rate of oviposition fell off less sharply, so that it needed about one-fifth of the oviposition period to produce about 35 per cent. of the total egg output. (Table XVI.) Canzanelli (1935) showed a marked decline in egg-laying on the third day of oviposition at approximately 23°C. Powell, working at 32°C., found just over a quarter of the eggs was laid on each of the first two days, and then the oviposition declined gradually for four days, by which time 98 per cent. of the total had been laid.

Rate of Increase.

Theoretical calculations.

Sufficient data have been accumulated in this paper for the calculation of the theoretical rate of increase of this species at several temperatures, at 70 per cent. R.H., with wheatfeed as food. An approximate method of calculating the intrinsic rate of increase is explained by Howe (1953), who estimated this parameter for *L. serricorne* using data obtained from the literature. He estimated that, at about 25–27°C., a stable population of *L. serricorne* was capable of increasing by about 60 per cent. every week. This figure fits extremely well with the results of the present work. These are presented in Table XVII,

TABLE XVII.

Theoretical rate of increase of a stable population on wheatfeed at various temperatures, at 70 per cent. R.H., and data on which calculations are based.

Temp. (°C.)	Working units (days)	Devel. period in units	Survival(%) Egg Other stages	Effective ♀ egg no. per ♀	r per unit	r per week	% increase per week
35	1	32.25	73 93	31.6	0.107	0.750	112
30	1	33.5	70 97	25.4	0.097	0.676	97
30N	1			19.8	0.089	0.624	87
25–27H	7	7	90	28.8	0.480	0.480	62
25	2	25.75	73 100	34.7	0.138	0.482	62
22.5	2	33.5	74 97	26.4	0.098	0.342	41
20	4	30	51 83	1.7	0.018	0.031	3

N, No water supplied.

H, Howe (1953).

together with the basic data from which the estimates of the rate of increase were made. The method of calculation is illustrated in Table XVIII, using the results for 35°C. Oviposition was completed in ten days. The oviposition period must be divided into 2, 3, 5, 7 or 11 equal working units which should be the shortest convenient units. Here, by adding at the end one day in which no eggs were laid, we can divide the oviposition period up into 11 units of one day each as in Table XVIII. The values for the daily oviposition rate given in Table XVI are divided by two to give the female egg-number, since there is no evidence that the sex ratio is not unity. The daily egg-number is further corrected to allow for the developmental mortality shown in Table XVII. This was estimated from all the experimental results available in the present work, most of which are shown in Tables III and XV. The developmental period measured to the middle of the first oviposition period was estimated from Tables I, III and IX. The computational method is iterative, but usually gives a satisfactory result in two attempts. From the result given by Howe (1953), $r = 0.48$ per week, it can be judged that with a working unit of one day, the intrinsic rate of increase, r , per day, will be between 0.05 and 0.1, probably closer to the higher figure. Therefore we copy the column of weighting values relating to $r = 0.1$ from the table given by Howe (1953), as shown in Table XVIII, and multiply the corrected egg-number for each oviposition period unit by its appropriate weighting factor, adding together the eleven products as shown. The sum of the products is divided by the weighting factor of the first period and the result, 31.6 eggs, is the total number of eggs, which, if laid during the first period, would enable the population to grow at the same rate as the observed pattern of oviposition. The value of r is now obtained by dividing the natural logarithm of the egg-number by the

developmental period, as shown in Table XVIII. In this example the value obtained for r was 0.107, which is close to the value 0.1 assumed for weighting purposes, so this value is accepted. If the calculated value had differed from that used for weighting by more than about 0.025, a second calculation would be needed using a new set of weighting values applicable to the calculated value of r , and obtained by interpolation from the table given in Howe (1953).

TABLE XVIII.

Method of calculating the intrinsic rate of increase, r , using values for 35°C.

Laying period	Raw egg number	Multiplied by the correction factor for sex and mortality [= 0.34]	Weighting factor (from Howe, 1953, Table 1E)	Product
1	13.2	4.49	14	62.86
2	28.3	9.62	13	125.06
3	17.9	6.09	12	73.08
4	16.1	5.47	11	60.17
5	11.8	4.01	10	40.10
6	11.2	3.81	9	34.29
7	11.0	3.74	8	29.92
8	5.3	1.80	7	12.60
9	1.8	0.61	6	3.66
10	0.3	0.10	6	0.60
11	0	0	5	0
				442.34

The above egg pattern is equivalent to a total oviposition of $442.34/14 =$

31.60 in the first laying period.

Developmental period to middle of first oviposition period is 32.25 days.

Therefore $32.25 r = \log_e 31.60 = 3.45303$

whence $r = 0.1071$ per day.

The value obtained for r , the intrinsic rate of increase per day, can be converted to a value of r per week by multiplying by seven, giving a value of 0.750. This, in turn, can be changed into a simple self-multiplicative rate of increase, λ , by finding its antilogarithm from tables of natural logarithms, because $r = \log_e \lambda$. In this instance, $\lambda = 2.12$, that is, in these constant conditions, a stable population of *L. serricorne* can increase 2.12 times or by 112 per cent. every week.

The rate of increase falls with temperature (Table XVII), so that at 22.5°C. it is only 1.41 times a week. Down to 22.5°C. the oviposition rates given in Table XVI have been used. This Table omits non-laying and infertile pairs, assuming one member to have been injured when sexed. At 20°C. it is probable that low temperature is a cause of oviposition failure and the egg-number from Table XV has been converted to Table XVII into eggs per female, including non-layers, by dividing by five. When this is done, the species is found to increase by 3 per cent. per week at 20°C., whereas the value on the original figures would have been 14 per cent.

The availability of drinking water at 30°C. slightly increases the rate of increase (Table XVII).

Warehouse data on rate of increase.

In the field it is unlikely that a stable population ever exists, but the concept just considered is of value in defining the maximum rate of increase possible in

given conditions. In temperate countries, breeding is restricted to the warmer part of the year and the yearly cycle probably starts with overwintering large larvae only. As a result, there is a spring emergence of adults, followed, later in the year, by further emergences of adults of succeeding generations. In these circumstances, with short-lived adults, the figures for rate of increase apply fairly accurately, when there is no great overlap of generations. In the tropics, where continuous rapid breeding is possible, the normal rate of increase on most food-stuffs would quickly make control measures very necessary. Thus 500 larvae in a bale of tobacco is considered a heavy infestation requiring treatment.

There are few references to the rate of increase in warehouses or in similar conditions. Jepson (1939*b*) found that one pair of *L. serricorne*, protected from natural controlling factors, produced 2,000 individuals in four months. This implies an increase in numbers of about 50 per cent. weekly, equivalent to an r of 0.4. In Mauritius, a pair normally produces a maximum of 500 in this period, that is, an increase of 38 per cent. weekly and an r of 0.32. Smeë (1923) estimated that a 25-fold increase in three months was a low estimate for Nyasaland. This is an increase of 28 per cent. weekly and an r of 0.25. Later (1945), he found that 53 individuals on local cigars multiplied to 827 in ten weeks, an increase of 32 per cent. per week, equivalent to an r value of 0.27. This latter value also fits the observation of Reed, Morrill & Livingstone (1934), who recorded a threefold increase during the month of August. A slower rate was found in Nigerian cocoa by Riley (1957), who noted an increase from 200 to 6,400 in nineteen weeks, a 20 per cent. weekly increase with $r = 0.18$. Ustinov (1932), collecting from warehouse windows in the Caucasus, found peak numbers in the ratio of 1, 15, 20 in June, August and September. A 15-fold increase in seven weeks is equivalent to nearly 50 per cent. increase per week with r being about 0.39, and this was achieved while temperatures were rising from about 25° to 28°C. Tirumalarao & Nagarajaroo (1955) obtained an increase on ginger stored in the laboratory of only 21 to 84 in thirteen weeks, about 11 per cent. increase per week.

Number of annual generations.

In the literature, references to the speed of increase of the species are usually expressed as the number of generations noted in a year. In the laboratory, at 30°–35°C., the average length of a generation was about 33 days; at 25°C. it was about 50 days and at 20°C. about 120 days, that is, from eleven down to three generations a year. Bovingdon (1931) records five to six, and Runner three to six generations annually in their cultures. In the field the highest number of generations quoted is seven, by Wille (1931) for Peru, made up by five summer generations and one or two winter generations. Jepson (1939*a*) gives four to five generations for Mauritius, Delassus & Lepigre (1931) three to six for Algeria, Stamatinis (1935) and Ustinov (1932) three for Macedonia and the Caucasus, respectively. Runner also counted three generations a year in Tennessee and Virginia, U.S.A., and estimates that there should be three to six farther south. In Southern Rhodesia there are just over two complete generations a year (Mossop, 1937), in Bulgaria, two (Mokrzhetzki, 1925), and in Japan, one to two (Shibuya & Yamada, 1935). In Britain, there is only one generation a year in warehouses (Bovingdon, 1931), and Canzanelli (1935) found only one per year in Italy, but he noted a wide overlap of stages, presumably because the mild winter does not kill many individuals. These reports are in good accord with the laboratory observations, for, in Peru, Mauritius, Greece, Italy and the U.S.A., outdoor temperatures can rise to a daily mean of 27°–28°C., and in parts of Peru and in Mauritius the mean daily outdoor temperatures do not fall below 20°C. In all the other countries mentioned, the mean daily temperature does fall below 20°C., but only in Britain and the Caucasus are the winters cold enough to cause

much mortality, although in the other places breeding may be arrested for about five months. The mean daily temperature does not exceed 21°C. in Southern Rhodesia and in the Caucasus it does not exceed 22°C. In all places, of course, it is warehouse and factory temperatures which are important, and these may vary from factory to factory, but outdoor conditions indicate the minimum possibilities for increase.

Limiting conditions.

The conditions in which development of *L. serricorne* was possible in the experiments described here are shown in fig. 7. These are interesting because the lower humidity limits differ with temperature, mortality being low at low humidity only at 30°C., and because these humidity limits are lower than any previously stated in the literature. Thus Powell (1931) puts the lower humidity limit at 45 per cent. R.H. but Canzanelli (1935) puts it at 30 per cent., at which humidity Powell obtained no development. Fraenkel & Blewett (1943a) failed to rear *L. serricorne* on flour at 40 per cent. R.H. at 25°C. High humidity does not prevent the growth of *L. serricorne* unless the food is destroyed by mould growth. The maximum temperature at which development was completed varied from 35° to 37.5°C. according to humidity. Runner (1919) states that 36°C. is fatal, but Powell (1931) obtained complete development at this temperature. The developmental minimum is just below 20°C. Powell obtained very high mortality at 22°C. The influence of temperature on distribution is thus fairly clear. The beetle can be expected to multiply whenever the temperature exceeds about 19°C. in infested produce either out-of-doors or in a warehouse. It is possible but unlikely that it is ever too hot for *L. serricorne* except in direct sunlight. The maximum outdoor temperature in some parts of the world may exceed 35°C. every day for some months, but the temperatures inside warehouses seldom reach this except in produce "heating" because of insect activity. In the hot areas it is possible, however, for it to be too dry for *L. serricorne*, especially in tobacco, groundnuts and foods of other low moisture content. Even for food such as wheatfeed and cowpeas, very dry areas must be considered marginal for *L. serricorne* on the basis of these laboratory observations. It can be reasonably assumed that the moisture content of foods will equilibrate approximately with the average relative humidity of an area, so that a mean relative humidity below 25-30 per cent. could be expected to prevent the breeding of *L. serricorne*. It is surprising, therefore, to have reports of *L. serricorne* being found during the dry season in the drier areas of northern Nigeria (Hayward, 1954). If these specimens were really subjected to the ambient humidity of the area, some stages in the life-cycle must be very resistant to low humidity. Unfortunately no details of moisture content or storage conditions are available concerning the infested produce (cowpeas). In Kano, the average relative humidity falls to 22 per cent. in March and is below 35 per cent. for six months from November onward.

Some of the statements in the literature do not agree with the observations made in this work. Delassus & Lepigre (1931) state that the maximum temperature for this species is 47°C. It is stated that specimens will live at temperatures up to 50°C. (Thillard, 1921; de Bussy, 1917b) or even at 55°-60°C. (Veth & van Rijn, 1915) without any reference to how long they will live at these temperatures or survive following the exposure. Probably the insects were not exposed to these temperatures but were inhabiting the cooler zone around the periphery of a fermenting heap of tobacco which reached these temperatures internally.

In the present work a few of the developmental stages were exposed for one week to 40°C. and 70 per cent. R.H. The experiment was set up at 30°C. and 70 per cent. R.H. Ten larvae were placed at 40°C. for a week as soon as they hatched, and ten more at 7 days old and a further ten at 14 days old. Further

individuals were put at 40°C. after pupation. The number of larvae dying during the week at 40°C. decreased with age but the length of the developmental period increased with age at exposure. In each group the shortest developmental period for a larva was 25 days, i.e., 18 days at 30°C. and 7 days at 40°C. It seems possible, therefore, that some individuals are not much affected by a week at 40°C. although apparently little, if any, growth takes place, whereas others die if exposed when young but survive if older, needing some time to reverse the deleterious

TABLE XIX.

The larval period and mortality of *Lasioderma* larvae and pupae reared on wheatfeed at 30°C. and 70% R.H. and exposed at 40°C. and 70% R.H. for one week.

Age at exposure (days)	No. surviving	Larval period (days)			No. dying
		M	±	S.E.	
Control	5	17.2		0.49	0
0 (hatching)	5	26.6		1.75	5 + 1 pupa
7	7	28.3		1.30	2 + 1 pupa
14	8	31.9		2.59	0
As pupae	1	—		—	14

effects of high temperature. Of the pupae exposed to 40°C. only one emerged as an adult from the cocoon. Three other pupae moulted, but the adults that were formed died in the cocoon at 40°C. and ten pupae reached the adult stage but failed to cast the pupal skin. The results are summarised in Table XIX.

No examination was made, in this work, of the effects of temperatures only just below the developmental threshold, and there is little in the literature on this subject other than the observations made on overwintering. Skalov (1931) observed hatching in eggs exposed to a range of temperatures from 33°C. down to -4°C., showing that short exposures to very low temperatures are not lethal. He failed to obtain any hatching at 13.5°C., and Crumb & Chamberlin (1934) similarly failed at 10° to 15°C. Bovingdon (1931) puts the developmental minimum at about 15.5°C.

Seasonal abundance.

In tropical countries such as Cuba and the Philippines (Jones, 1913), *L. serripennis* is abundant throughout the year. Thillard (1921) states that this beetle is most abundant in the dry season in the Cameroons, and Jones (1913) found adults most frequently in March–April, the driest and also the hottest months, in the Philippines. Runner (1919) and Tenhet & Bare (1951) discuss seasonal changes of abundance in the southern States of the U.S.A. Runner states that genuine seasonal changes are masked by changes in the amount of food supply and by influxes of individuals with imported Cuban tobacco. Otherwise adults are most abundant in Florida in February–March and again in August–September, but in Virginia and Tennessee the adult is not really abundant until June, with a second phase of abundance in September. In Tennessee, adults are rarely found from November to April. Delassus & Lepigre (1931) and Melia, Melia & Lepigre (1934) find the adult is scarce in Algeria between October and April. Runner based his estimate of three annual generations in Virginia on an experiment in which he put eggs on tobacco in a warehouse in October and obtained adults in May, July and October. Tenhet & Bare (1951) record adult appearances in May, July and September for Virginia and North Carolina. These correspond closely with the dates given for Greece by Stamatinis (1935). In Southern Rhodesia (Mossop, 1949), a spring emergence of adults appears in September–October, a second

generation in December-January after which the generations overlap and there are no further peaks. Reed, Morrill & Livingstone (1934) trapped adults in an open store in the bright tobacco belt of the U.S.A. between August and November, using light-traps with reflectors and continuously operating suction devices. The catches in these, which reflect activity as well as abundance, were closely correlated with temperature but were unaffected by humidity. The peak catch was made in the last week of August when the average minimum temperature was 23°C. (mean 26°-31°C.). Very low catches were made after mid-October when the highest daily minimum temperature was about 17°C. and the mean varied from 11° to 20°C. There was an abrupt fall after the first week in September, associated with a fall in maximum and mean temperatures. In a closed warehouse examined from mid-September to early November, temperatures were higher and the best catch was made in the first week of October when 74,000 beetles per trap were caught. The mean temperature was only 18°C., but the range was 11° to 24°C. Activity was possible only during the warmer hours when, in an open store, daylight would have prevented activity, but, here, since the store was kept closed and dark the adults flew and were caught. This work was repeated (Reed, Morrill & Livingstone, 1935) over the months May to November. The peak catches in four open warehouses were obtained over a period extending from late July to late September. In a closed warehouse three peaks were noted, one in June, another in late July to early August and a larger one in early September, corresponding to brood emergences. They note that catches are small in the spring and increase rapidly in August and September. This they attribute not to multiplication in numbers from one brood to the next but to a greater degree of migration by adults of the second and third broods as compared with the spring brood. Throughout this period the mean temperature was about 22.5° to 26.5° and the minimum about 16° to 20°C. in all the buildings.

Riley (unpublished), in Nigeria, followed the increase of *L. serricorne* in cocoa stores from December to May, by counting the numbers of adults trapped in soap solutions and on sticky strands. The change was approximately exponential and fairly similar in four stores, but showed some signs of the appearance of three distinct generations in this time. The increase noted was from about 2 to 5 insects trapped per count per store in December or January to 50 to 400 in April or May.

The Trapping of Adults.

Light-traps have been extensively used in tobacco warehouses, originally in the hope that they might give effective control (Reed, Morrill & Livingstone, 1934). Although they have proved ineffective for this purpose they have at least indicated the size of populations of *L. serricorne* in warehouses. Thus Reed, Morrill & Livingstone (1934) used up to 14 light-traps, each fitted with reflectors and a continuously operating sucking device, in a warehouse of 2½ million cubic ft. capacity, and caught 1¼ million adult beetles with a best average of nearly 100,000 per trap in 3-4 days. Such large catches are estimated by volume (Brubaker & Pollard, 1941), and illustrate how small a proportion of adults are caught by other methods. Thus, in a tobacco warehouse Reed, Morrill & Livingstone (1934) caught a maximum of only 611 adults per sheet using boxes lined with fly papers and containing a light, with a maximum weekly catch of 1,856 in any one trap. Golding (1941) caught 658 adults in a fortnight on sticky strands in a Nigerian cocoa warehouse. Runner (1919) and Ustinov (1932) note that adults may be collected at windows. Trapping as a means of control has been extensively advocated. Delassus & Lepigre (1931) suggest light-traps with 15-candle-power bulbs and sticky paper. Smee (1923) used fly papers, Jack (1938) mentions the practice of smearing engine oil on windows, Melia, Melia & Lepigre (1934) put translucent sticky paper on windows, and Reed, Morrill & Livingstone (1934) mention such

practices as smearing windows and skylights with cylinder oil, castor oil, or tangle-foot and cup grease. Runner (1919) and Jones (1913) captured beetles by hanging hands of leaf tobacco in warehouses, and de Bussy (1917a) proposed the use of wide-mouthed bottles baited with tobacco leaves or cigars.

Exclusion of Adults from Premises and Products.

Local spreading occurs in the tropics where the adults can fly in the open, so the exclusion of adults from buildings and produce has been attempted. Originally it was suggested that warehouses should be covered with mosquito netting or cotton fabric (de Bussy, 1917b; Jensen, 1917) and later it was recommended that doors, windows and open eaves should be covered by fine-mesh wire gauze (Smee, 1923; Delassus & Lepigre, 1931; Melia, Melia & Lepigre, 1934). Vinzant & Reed (1941) specify the standard type of U.S. mesh required, recommending one as fine as 20 meshes to the inch. Corbett (1931) noted that books kept in cases fitted with wire gauze were much less infested than books not so protected. Attempts have also been made to exclude *L. serricorne* from produce by using insect-proof containers or covers. Reed, Brubaker & Pollard (1941) found that hogsheads for tobacco could not be built so as to exclude this species because gaps must be left to allow ageing and fermentation. Bissell & Du Pree (1946) recommended the use of cotton sacks and Strong (1936) of paper sacks to reduce the liability to infestation by this beetle. Annand (1942b) states that lining hogsheads of tobacco with paper gives effective protection, and Chorley (1944) attributes an increase in the incidence of *L. serricorne* to an enforced wartime reduction in the use of wrapping paper for bales of tobacco. Mossop (1932) noted that beetles readily emerged outwards through brown paper but they did not penetrate inwards through it. Van der Veen (1940) also made this observation but noted further that adults laid eggs either on the outside of the cover, or if this were thin enough, through it. Cloth was preferred to paper as an oviposition site. Newly hatched larvae were able to penetrate the heavier cloths and paper but, since the distance such young larvae can wander is limited, he suggested enclosing the stacks with light cloth and adding an outer covering of brown paper, the whole stack being kept off the floor.

Means of Spread of *L. serricorne*.

Local spreading of *L. serricorne* from one warehouse to another was observed in Mauritius by Jepson (1939a) and in the U.S.A. by Reed, Brubaker & Pollard (1941). Freeman (1950) found that adults were blown by the wind from an infested cargo of cottonseed cake being unloaded at a British port on to previously uninfested flour on the quay. In Greece and Turkey, Reed (1935) observed that new and old crops were often stored in close proximity and that this encouraged cross-infestation. Freshly cured tobacco was quickly infested by *L. serricorne* in rural Greece. Attempted migration out of warehouses is indicated by the presence of adults on the inside of windows at dusk. Ustinov (1932) records finding a thousand or two on windows and sills in summer.

There are many statements in the literature on the types of tobacco which are most attractive to *L. serricorne*, but no attempt is made here to analyse these statements. Morgan & Crumb (1928) carried out some laboratory experiments to find out which substances might attract adults of this species. Substances which proved to be definitely attractant included boiled linseed oil, tobacco, yeast cake, starch and peptone mixture, and starch. Nicotine, coumarin and agar agar were only slightly attractant. Powell (1931) showed that tobacco, yeast and furniture materials attracted adults of *L. serricorne*. Stamatinis (1935) considered that the odour of tobacco increases with age and correspondingly becomes more attractive to adults of *L. serricorne*. According to van der Veen (1940), young

larvae are unable to perceive tobacco at only a few centimetres distance and reach it only by chance.

On a wider scale *L. serricorne* is spread mainly by trade. Hayward (1954) discusses its spread on cowpeas in Nigeria.

Jones (1913) noted its presence on cars, boats and other vehicles in the Philippines. Brannon & Reed (1943) failed to find it in rail or road trucks but they did find it in most of the tobacco imported into the U.S.A. They also noted the shipment of other infestible produce with tobacco. Runner (1919) mentions the influx of *L. serricorne* into the U.S.A. in Cuban tobacco, and Freeman (1948) and Howe & Freeman (1955) give examples of its introduction into Britain on foodstuffs. Runner (1919) discusses the respective importance of trade and local spreading to the tobacco industry of the U.S.A.

Damage.

Damage to stored produce may cause loss of weight, of quality and of reputation. The loss of weight due to a single individual is very small, only a few milligrammes, but with populations measured in millions this would be a significant amount. Even so, the loss of quality is probably more important. Produce is holed and contaminated with cocoons and frass. In cigars and cigarettes the holes ruin the product, while, in other produce, holes spoil the sack or package. The problem of insects penetrating into packeted foods is discussed by Linsley (1944) and the importance of injury by *L. serricorne* to tobacco is considered in numerous papers. Contamination of human food by insect fragments and frass is the subject of legislation nowadays so that the discovery of even a small amount of such contamination could prevent the sale or importation of infested produce. Nevertheless, Doane (1925) established that the feeding of infested copra meal to cows caused no ill effects and that the infested material was of equal food value.

Infestation of cereal grains and of seeds of beans and other plants could affect germination when, as Fraenkel & Blewett (1943a) record, the germ is the region attacked.

A serious risk with *L. serricorne* is that a huge population will build up quickly and cause considerable damage. Such an infestation was carefully examined by Back (1939b) in cottonseed cake stored both in bags and in bulk. The insects penetrated into the bagged stack. In the outer two to three inches of the bagged material and in the outer six inches of the bulk he found nearly 7,000 individuals per U.S. pint. In refuse meal on the floor he found 6,000 per U.S. pint. On the inner surface of the sacking there were 225 cocoons per square inch. Back assessed damage in this example in terms of sacks lost, because such an infestation weakens and breaks sacks, the cost of labour for regrinding and resacking, fumigation costs, discount on returned material and the effects on trade reputation.

In tobacco, Ustinov (1932) estimated that 40 per cent. of leaves were damaged and 2 per cent. of leaf surface damaged. Delassus & Lepigre (1931) put losses at 5 per cent. Stamatinis (1935) states that damage can reach 5 per cent. in a year and exceptionally can reach 50 per cent. In Nigeria, Golding (1941) estimated that 5.8 per cent. of cocoa beans were attacked. Runner (1919) gives a good description of the nature of damage to cigarettes and tobacco and discusses financial losses. A more modern statement about these points is given by Tenhet & Bare (1951).

The Lethal Effects of High Temperatures.

Tobacco is subjected, during processing and manufacture, to high temperatures sufficient to kill any *L. serricorne* in the treated material. Hence manufactured tobacco should start free of infestation and would remain so if it could be stored in

TABLE XX.

High temperatures and exposures thereto which cause complete mortality of
L. serricornis, according to various authorities.

Temp. (°C.)	Period stated	Remarks	Authority
84	20 min.	All stages, 100% R.H.	P
82.5	15 min.	Eggs on leaf tobacco	R
77-80	16 min.	Vacuum 27.2 in. of mercury	B+
77	6 min.	All stages, 100% R.H.	P
70-73	30 min.	Eggs on leaf tobacco	R
71	20 min.	All stages, steam in closed drum	R
42-100	20 min.	All stages, 100% R.H.	P
65-70	60 min.	—	Mad
63-71	45 min.	All stages on leaf tobacco	R
65	5 min.	All stages	Car
35-95	50 min.	All stages, 100% R.H.	P
65	60 min.	—	R
65	2 hr.	Heating whole room	Cr
62.5	1 hr.	All stages on leaf tobacco	R
60-63	6 hr.	Heating whole room	Cr
60	5 min.	Eggs, 75-80% R.H.	C
60	3-5 min.	Pupae, "	C
60	8-10 min.	Larvae, "	C
60	15 min.	Adults, "	C
60	40 min.	All stages, steam in drum	R
60	1-2 hr.	All stages, books, etc., in oven	T
60	105 min.	With bundles leaf tobacco	St
60	2 hr.	All stages	Sk
60	24 hr.	In furniture stuffing	W
60	—	—	DL
60	20 min.	Steam in drum, 4 atmospheres	J
60	—	Eggs in steam blast	Mor
59-60	½ hr.	Eggs, larvae in cigars	R
59-60	1 hr.	Pupae in cigars	R
59	1 hr.	All stages, steam in drum	R
59	2 hr.	All stages, box of tobacco	R
58	24 hr.	Steam-heated room	R
55-60	5 hr.	All stages in bale of tobacco	A
54.5-60	2 hr.	—	R
55	10 hr.	Eggs, larvae in cigarettes	St
55	5 hr.	Adults	A
53-55	1 hr.	Eggs in tubes	R
54.5	5 hr.	Heating whole room to 65°C.	Br
51.5-54	10-12 hr.	—	G
51.5	24 hr.	All stages on hessian and paper	Mos
50-51	15 hr.	Eggs in cigarettes	St
50	16½ hr.	—	Sk
50	8½-12½ hr.	Pupae, 75-80% R.H.	C
50	5 hr.	Bales in air at 60°C., 65% R.H.	Sc
50	5 hr.	Adults, larvae, eggs	A
50	2 hr.	Larvae	A
48-50	72 hr.	All stages	Gh
48-50	24 hr.	Larvae	Sk
48-50	17 hr.	Adults	Sk
48-50	14 hr.	Adults in tubes	St
36-60	24 hr.	All stages	R
46-50	24 hr.	Pupae	Sk
47	continuous	Adults	R
45	30 min.	All stages	Car
44	10 days	All stages, 62% R.H.	P

Abbreviations: A, d'Angremond, 1919; B+, Bare, Tenhet & Reed, 1946; Br, Britton, 1920; C, Canzanelli, 1935; Car, Carloni, 1950; Cr, Cressman, 1933; DL, Delassus & Lepigre, 1931; G, Gowdey, 1923; Gh, Ghimpu, 1935; J, Jones, 1913; Mad, Madel, 1938; Mor, Moreira, 1923; Mos, Mossop, 1937; P, Powell, 1931; R, Runner, 1919; Sc, van Schreven, 1948; Sk, Skalov, 1931; St, Stamatini, 1935; T, Tucker, 1934; W, Wallace & others, 1926.

insect-free conditions. There has therefore been a great deal of investigation of the effects of heat on *L. serricorne* and also some on the effects of cold, in the hopes that the use of cool storage would prevent or reduce damage by this insect. In measuring the effect of high temperature, the important factors are the temperature to which the insect is exposed and the duration of exposure. The length of exposure is often omitted from accounts given in the literature. Indeed, if the insect is exposed inside a mass of produce, it may not be easy to measure either temperature or exposure because of the time required for heat to penetrate into the produce. This was realised by Stamatinis (1935) and Carloni (1950) who quote the temperature and exposure necessary to kill the insect but add that the time needed for the produce to heat up must be added to the exposure time. From the developmental data given earlier, it is clear that if temperatures above 38°C. are maintained indefinitely a population of the species must die out. De Bussy (1917b) is clearly mistaken in the suggestion that temperatures up to 50°C. do not hinder development. From the data given in the literature, which is summarised in Table XX, it appears that about 24 hours at 50°C. and a few minutes at 60°C. will kill all stages of this species. The figure of 30 minutes at 45°C. given by Carloni seems too low, for Skalov (1931) obtained only 50 per cent. mortality of eggs in 24 hours at 48°C. and larvae in 22 hours at 45°-49°C., respectively. Stamatinis (1935) also had survivors among eggs and larvae exposed in cigarettes to 45°-50°C. Canzanelli (1935) recorded 70 per cent. mortality of eggs in two minutes at 60°C. Some of the periods stated in Table XX are long, and evidently include the time required in practice to kill insects which have burrowed deep into the produce.

Duport (1919) considers that dry heat kills more quickly than damp heat.

The Lethal Effects of Low Temperatures.

If produce can be stored below the developmental minimum of the species, about 18°C., it should be safe from attack by *L. serricorne*. This should be easy in temperate areas but in hotter places it would require refrigerated storage. Runner (1919) noted that adult activity ceased when the temperature fell below 18°C. and Annard (1942a) records that storage is safe at temperatures in the region of 15°-18.5°C. At 8°-14°C., Jones (1913) found that pupae transformed into adults which could live over 100 days, but larvae did not feed. On the other hand, Crumb & Chamberlin (1934) found that large larvae, which could live for 45 days at 10°-15.5°C., did hole cigars. They found that eggs were not viable after 35 days and newly hatched larvae died within 21 days at these temperatures, and that at 18°C. very few larvae survived after exposure for 32 days as eggs or as larvae. Skalov (1931) found that eggs were not viable after 33 days at 10°C. Delassus & Lepigre (1931) allege that the beetle can live for hours at -18°C. but Bovingdon (1933) had only one adult out of nine survive 114 minutes at -6° to -7°C. *L. serricorne* cannot survive the winter in England, where Mansbridge (1936) found that it had died by February in a year when the temperature fell to 2°C., nor in Rumania (Ghimpu, 1935) where the cold winters prevent its becoming established. In the Caucasus it usually survives in some areas, but during one year (Skalov, 1931) a month with temperatures in the range 0.4° to -9.3°C. killed all stages of the species. Solomon & Adamson (1955) record that *L. serricorne* failed to survive a much milder winter than that described by Mansbridge and that less than five per cent. of larvae survived a 16-day period having a minimum of -1°C.

Runner (1919) stated that the larva becomes dormant at temperatures below about 15°C. and does no damage but can survive long enough to pass the winter. Overwintering of the larva has been recorded in the U.S.A. (Runner, 1919), Japan (Shibuya & Yamada, 1935), Greece (Stamatinis, 1935), Italy (Canzanelli, 1935) where the minimum temperature recorded was 15°C., Bulgaria (Mokrzhetzki.

TABLE XXI.

Low temperatures and exposures thereto which cause complete mortality of
L. serricornis, according to various authorities.

Temp. (°C.)	Period stated	Remarks	Authority
-18	some hours		DL
14	14 days	In bales of tobacco	DB
12	1 hr.	Eggs, pupae, adults	Sw
11	4 days	In box of tobacco	R
10	8½ days	Larvae in bale of tobacco at -10.5°C.	Sw
10	7 days	All stages in box of cigars	R
10	28 days	All stages in bale of tobacco	R
10 to 9	4 days	Adults, larvae, pupae, in box of tobacco	R
9.5	3 hr.	Eggs, pupae	Sw
9.5	5 hr.	Adults	Sw
9.5	60 hr.	Larvae	Sw
9.5	3 days	All stages	TB
9	2 days	Pupae	Sk
9	1 day	Adults	Sk
10 to 8	24 hr.	Eggs	R
9 to 8	5 days	All stages	R
10 to 7	4-5 days	All stages	Z
8	6½ days	Adults in bale of tobacco at -10.5°C.	Sw
8	6 days	Eggs, pupae in bale of tobacco at -10.5°C.	Sw
8	4 days	All stages	J
10 to 5	3 days	Larvae	Sk
9 to 5	3 days	Eggs	Sk
6.5	1 day	Eggs, pupae	Sw
6.5	2 days	Adults	Sw
6.5	5 days	Larvae	Sw
7 to 6	17½ hr.	Adults	Bov+
10 to 3	22 days	All stages	Pk
4	7 days	All stages	TB
4	7 days	Eggs, larvae, pupae	Sw
4	6 days	Adults	Sw
5 to 0	5 days	Larvae, pupae, adults	Keu
2	7 days	All stages	Sw
1	11 days	Eggs, adults	Sw
1	14 days	Larvae	Sw
1	12 days	Pupae	Sw
0	14 days	Pupae	Sw
0	13 days	Adult	Sw
0	12 days	Larvae	Sw
0	11 days	Eggs	Sw
+2	12 days	Eggs	Sw
2	16 days	Adults, larvae, pupae	Sw, TB
4.5	16 days	Eggs	Sw
4.5	20 days	Larvae	Sw
4.5	33 days	All stages	Sw, TB
4.5	40 days	All stages	Str
7 to 10	4-5 days	—	Mad
10	33 days	Eggs	Sk
8 to 14	30 days	Young larvae	J
8 to 14	151 days	Large larvae	J
10 to 15.5	21 days	Newly hatched larvae	CC
10 to 15.5	35 days	Eggs	CC
13	21 days	Newly hatched larvae	CC
13	33 days	Eggs	CC

Abbreviations: Bov+, Bovingdon, 1933; CC, Crumb & Chamberlin, 1934; DB, Diakonoff & de Boer, 1938; DL, Delassus & Lepigre, 1931; J, Jones, 1913; Keu, Keuchenius, 1917; Mad, Madel, 1938; P, Pocock, 1910; R, Runner, 1919; Sk, Skalov, 1931; Str, Strong, 1936; Sw, Swingle, 1938; TB, Tenhet & Bare, 1951; Z, Zacher, 1927.

1925) and the warmer parts of the Caucasus (Ustinov, 1932). There is usually a heavy mortality of immature larvae during the winter (Tenhet & Bare, 1951).

An excellent account of the lethal effects of cold is given by Swingle (1938). The results of published accounts of the exposures to low temperatures required to kill *L. serricorne* are summarised in Table XXI and fig. 4. Where refrigeration is used, an allowance must be made for the time needed for the produce to cool. Swingle (1938) measured the rate of cooling of bales and hogsheads of tobacco stored at -12°C . Diakonoff & de Boer (1938) state that 14 days are needed at -14°C . for the centre of a bale to cool to this temperature.

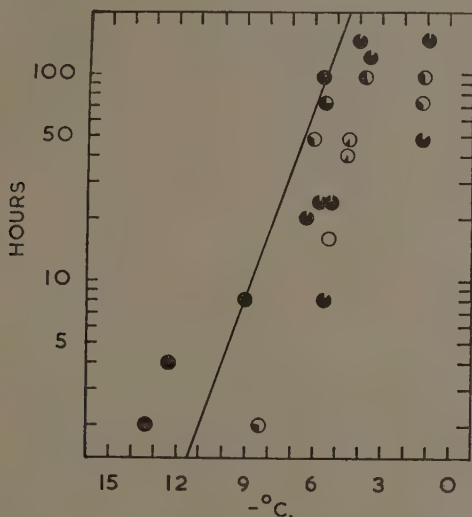


Fig. 3.—The survival of adults of *L. serricorne* exposed to cold for various periods. The proportion dying is shown by the area of black in each circle.

In the present work, the effects of cold on young adults only were examined and the results are presented in fig. 3. Swingle (1938) states that adults, as well as eggs and pupae, are less resistant than larvae at all temperatures below 2°C . but at 4°C . the resistance of all stages is more or less similar. Powell (1931) found that eggs survived 20 days at 2°C . which agrees with Swingle. Skalov (1931) reports surviving larvae after 6 days at -6° to -3°C . in which conditions, according to Swingle's results, survivors might be expected (fig. 4). Of the results included in fig. 4 only those of Keuchenius (1917) and Madel (1938) are anomalous.

Adults were exposed in this work in groups of five in glass tubes with no food medium. The tubes were placed in the ice box of a refrigerator and the temperature of each tube was measured several times, using thermocouples which were kept in place throughout each experiment. These gave a check of the range of temperature in each tube and on the variation between the tubes and enabled an approximate mean temperature to be calculated for each exposure. The extreme range noted for any experiment was 3°C . Humidity was maintained near to 70 per cent. by using a saturated solution of ammonium nitrate. At -13°C ., complete mortality was recorded in a two-hour exposure, and at -12.5°C . in a four-hour exposure. The only other tests to give a complete kill were an eight-hour exposure to -9°C . and a four-day exposure to -5.5°C . There

The mites mentioned are *Chortoglyphus gracilipes* Banks, by Banks (1917); *Pediculoides ventricosus* (Newp.), by Bare, Tenhet & Brubaker (1947), Canzanelli (1935), Livingstone & Reed (1936), Mossop (1932), Reed (1935) and Stamatinis (1935); *Cheyletus* spp., by Bare (1942) and Runner (1919); *Sciulus* sp., by Bare (1942) attacking eggs in a laboratory culture; *Monieziella angusta* Banks, by Bare (1942) which invaded cultures in a warm humid insectary and penetrated even into the larval cells, and *Rhagidia* sp., by Jones (1913) and Keuchenius (1917).

Parasites.

Nearly all the parasites recorded attacking *L. serricorne* are Hymenoptera of the families BETHYLIDAE and PTEROMALIDAE. The Bethylids include *Cephalonomia gallicola* (Ashm.) found by Kearns (1934) and also by van Emden (1931) who recorded it as *C. quadridentata* Duchaussoy. Both Kearns and van Emden give data on the biology of this parasite. Another Bethylid, *Israelius carthami* Richards, is described by Richards (1952). The commonest parasite is the Pteromalid, *Anisopteromalus calandrae* (How.), which has been recorded by Back (1939b), Bare (1942), Bare, Tenhet & Brubaker (1947), Livingstone & Reed (1936), Reed (1935) and Smee (1945). It has been recorded under the name *Aplastomorpha pratti* Crw. by Bodkin (1914), Kearns (1934), and Runner (1919) and under the name *A. vandinei* (Tucker) by Runner (1919). Cotton (1923) describes the biology of this species and Bare (1942) discusses the value of this and other natural enemies of *Lasioderma* in tobacco warehouses. Other parasites are the Eurytomid, *Bruchophagus* sp. found by Lever (1941), and the Pteromalids, *Lariophagus distinguendus* (Först.) mentioned by Bare (1942), Bare, Tenhet & Brubaker (1947) and Lever (1941), and *Chaetospila elegans* Westw. found attacking *L. serricorne* in laboratory cultures by Bare (1942). Jones (1913) and Keuchenius (1917) state that a species of *Norbanus* attacks larvae and pupae in their cells.

Conclusions concerning Predictions from Laboratory Data.

In an earlier paper (Howe & Burges, 1953) it was shown that the laboratory results for a temperate species, *Ptinus tectus*, which is still spreading, could be used to delimit, with fair accuracy, the areas of the world open to colonisation by the species. This kind of information would not be very useful for *L. serricorne*, a tropical species which has already spread throughout the tropics. It is still interesting, however, to compare the conclusions to be drawn from laboratory results with the observed facts. The most useful laboratory data given above are the rate of increase of the species on various foods, the physical limits, and the periods of exposure to cold required to kill *L. serricorne*. In the absence of a useful number of records of warehouse temperature and humidity, ordinary meteorological data have been consulted. Those available, for about a thousand stations, were the mean daily maxima and minima of temperature and humidity for each calendar month. The averages of the maxima and minima were taken as the means of temperature and humidity for each calendar month and were used as the basis for conclusions about the fate of *L. serricorne* at each station.

First the effects of cold were considered. If, at a station, the mean temperature in any one month was 40°F. or lower, or if 50°F. or less was recorded for five consecutive months, it was assumed that *L. serricorne* would be able to survive the period of cold only if protected by artificial heat. The position of stations where *L. serricorne* would be killed by cold, and of those where it would not be, were plotted on a map and by reference to these places, lines marking the limits of overwintering were drawn. These are shown on fig. 5 by thick lines marked W.

The developmental threshold of *L. serricorne* was taken as 65°F. The lines bounding areas in which the mean does not reach 65°F. in any month are shown

in fig. 5 as a line, marked T, bordered by cross shading. It will be seen that there are a few small areas such as Tasmania, parts of New Zealand and north-west Spain where the insect cannot complete its developmental cycle but dies only very slowly.



Fig. 5.—Map showing the probable maximum number of generations per year of *Lasioderma serricorne* when breeding on an optimal food. The figures show the number of generations likely at certain places and the lines mark off, approximately, areas with a uniform number of generations, subject to local modifications of climate due to altitude, etc.

For areas in which the temperature rises above 65°F., the annual number of generations on an optimal foodstuff such as wheatfeed or cowpeas has been estimated, and are shown in fig. 5. The estimates are based on laboratory data at 70 per cent. R.H. Most of the mean humidity readings at the various stations fall between 55 and 85 per cent. R.H., so that it is not likely that much error is introduced by this approximation. About a hundred stations experience one or more months with the relative humidity below 50 per cent. and in these places the number of generations stated in fig. 5 may be too high. The desert areas of the Sahara, Kalahari, Persian Gulf, India and Pakistan, Australia and the western United States all experience at least three dry months. Only those areas marked in fig. 5 with square shading experience conditions which fall outside the developmental limits of *L. serricorne* as shown in fig. 2. It has been assumed in drawing fig. 5 that no breeding is possible in these dry limiting conditions.

The maximum number of generations in a year for *L. serricorne* breeding at its fastest on an optimal foodstuff is 10½. The most recorded for any station was a little under 10, and for most of the tropics is 7 or 8. On a poorer food, such as tobacco, the number of generations must be reduced to two-thirds and on a still poorer food, such as cocoa, to only a half or less of the numbers given. It will be seen from fig. 5 that summer breeding is possible in Europe, Asia and North America in areas where overwintering is impossible. Here, introduced insects, or those protected in heated premises, can cause damage every summer. There are also areas where the conditions are not warm enough for one generation to be completed in a year and in most of these *L. serricorne* cannot pass the winter. Local variations of climate can cause marked differences in the rate of development of the species. Consequently in fig. 5 the figures representing the annual number of generations are drawn large where, in a large area, the number appears to be uniform, and small where there are local discrepancies.

The number of generations estimated for tobacco agree fairly well with some statements in the literature, namely Mauritius, four, Southern Rhodesia, two, and Italy, one; but are low for others. In the southern U.S.A., southern Europe and Russia, and Algeria there appear to be three generations per year as against the

estimated one or two. This underestimate is probably due to the storage buildings in these places being heated at times or it may be due to genuine underestimation for areas with average temperatures falling below 65°F. The method of estimating the number of generations is essentially a very rough temperature summation analysis, and Lin, Hodson & Richards (1954) have shown that if at any time the daily average falls a little below the threshold, a substantial amount of development, which does in fact take place, will be excluded. Atkinson (1886), however, does state that only two generations occur in North Carolina. For Peru and Japan the recorded seven and one to two generations on cotton seed and ginger root, respectively, are the same as given in fig. 5.

The most important difference between prediction and observation concerns the lower humidity limits. Hayward (1954) gives some details of captures of *L. serricorne* in northern Nigeria, one of the marginal dry areas for the species. He records its presence in Kano market in October and shortly afterwards, in cowpeas from Maiduguri, at Kano railway station. In November it was found in Kano and Ringim. These months in these places are damp enough to permit breeding but Nguru, where it was also found in November, is too dry for breeding, according to laboratory results. Hayward also records a heavy active infestation on cowpeas at Garin Gabbas from mid-February to June. The first six weeks of this period also fall below the lower humidity limit obtained in the laboratory. It seems quite possible that early in the dry season, soon after harvesting, the cowpeas had retained enough moisture to raise the humidity in the stack, but this apparent breeding after three months of the dry season cannot be explained in this way, for there is no obvious reason why the cowpeas should retain their moisture for so long. It is likely that only the egg and young larvae are vulnerable to low humidity and that the later stages may survive several months at low humidity.

In discussing the climatic preferences of *L. serricorne*, Hayward notes the scarcity of the species in Ibadan between June and August. This he attributes partly to the small quantity of cocoa in store at the time but also, especially in the tobacco factory, he suspects that it is due to the prevailing high humidity. This seems unnecessary, since the temperature is then at its lowest, so that activity and the rate of breeding are both at a minimum. On tobacco at this time a complete life-cycle would require about 85 days as compared with 55 days in February and March.

A few calculations were made of the maximum number of offspring produced in a year from a single female on an optimal food. For places in which continuous breeding is possible, the values obtained were so large as to be meaningless; in Ibadan, for instance, the average rate of increase possible is 70 per cent. per week. In temperate areas, the figures per year are more comprehensible and include about 200 offspring from one female for Buenos Aires, 675 for Japan, 925 for South Carolina, 1,250 for Southern Rhodesia, 2,800 for Algeria, and 7,500 for Greece. Even these increases are curbed by the action of parasites and predators, control measures, limitation of food and so on, but they illustrate the potential increase possible for this species.

Summary.

The paper includes a review of the published literature on the biology of the Cigarette Beetle, *Lasioderma serricorne* (F.). A list is given of foods on which this species has been found breeding. Information on the duration of the various developmental stages is tabulated and the data on oviposition are summarised. Observations are collected together on the rate of increase of the species in warehouses, on the number of generations per year, on sex ratio, on the use of traps to catch adults and on the spread of the pest and the damage that it causes. The lengths of exposure to various high and low temperatures that are required

to give a complete kill of the species are tabulated, and the known natural enemies are listed.

Original work includes a morphological comparison of *L. serricorne* and the closely related common storage species *Stegobium panicum* (L.). The experimental work consists of a study of the life-cycle of *L. serricorne* on wheatfeed over a wide range of combinations of constant temperature and humidity. Development was possible from just under 20°C. to 37.5°C. at favourable humidities. Larvae died at 90 per cent. R.H. at 37.5°C. The lower humidity level for development was under 25 per cent. R.H. at 30°C. but change of temperature, both up and down, raised the lower humidity limit so that, at 20°C. and 37.5°C., all larvae died at 40 per cent. R.H. When fed on foodstuffs such as groundnuts, which contain less water than wheatfeed at all humidities, the effects of low humidity are more rigorous. The effect of a number of foodstuffs on the length of the developmental cycle was investigated. The duration of larval instars on wheatfeed at 30°C. and 70 per cent. R.H. was found. The adults obtained in the experiments were weighed. The heaviest insects were obtained at 25°C. and 100 per cent. R.H. Higher temperatures and low humidities reduced weight. Females were heavier than males. The sex ratio was unity.

The oviposition rate was investigated at 70 per cent. R.H. over a wide temperature range. Under all conditions the eggs were laid quickly. At this relative humidity, no fertile eggs were laid at 37.5°C. and few pairs laid at 20°C. The total number of eggs laid per female at 20°C. was low, otherwise temperature had little effect on the number of eggs laid, but the rate of oviposition was increased by high temperature. The theoretical rate of increase at 70 per cent. R.H. was calculated for several temperatures and shown to increase with temperature up to 35°C.

The conclusions on the distribution and multiplication of the species which can be drawn from the laboratory results are discussed.

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FIG. 1. Antenna of, (a) *Lasioderma serricorne*, (b) *Stegobium paniceum*.

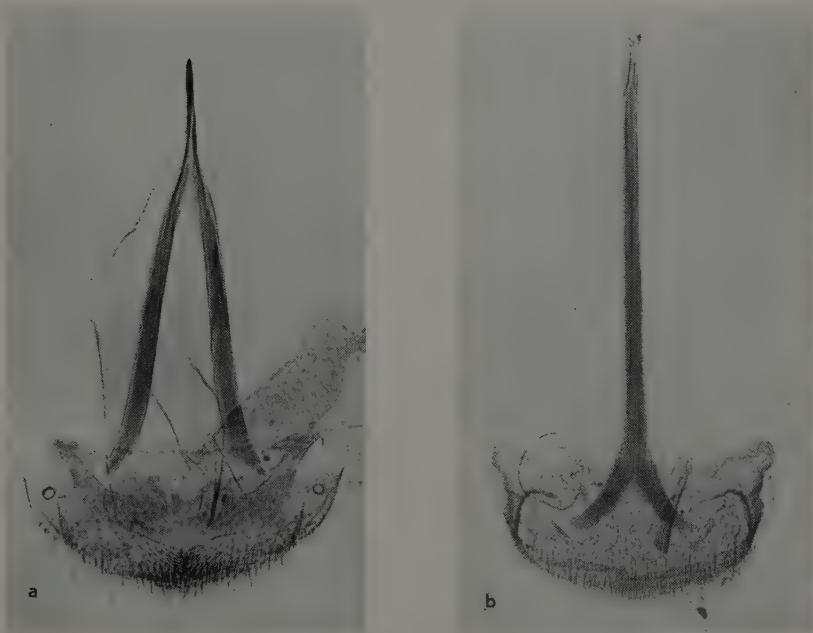


FIG. 2. Apodeme of female genitalia of, (a) *L. serricorne*, (b) *S. paniceum*.



FIG. 1. Ovipositor of, (a) *L. serricorne*, (b) *S. paniceum*.

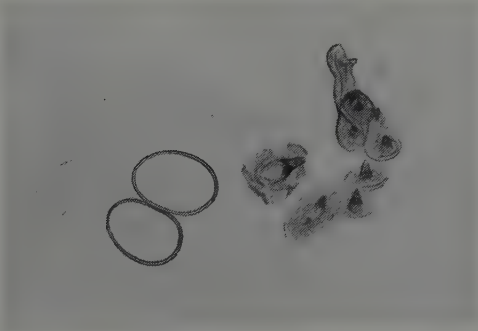


FIG. 2. Bursa copulatrix of *L. serricorne*.



FIG. 3. Aedeagus of, (a) *L. serricorne*, (b) *S. paniceum*.

TWO NEW SPECIES OF *PSEUDODONIELLA* CHINA & CARVALHO
(HEMIPTERA, MIRIDAE).

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The genus *Pseudodoniella* was proposed by China & Carvalho (1951) for the species *pacifica* from Keravat, New Britain. Recently two more species have come to hand, from Lae, New Guinea and Popondetta, Papua. They are new and are described and figured here. It has been found that in the genus *Parabryocoropsis* China & Carvalho (1951), two of the species then described, *cheesmanae* from Kokoda, Papua and *duni* from Keravat, New Britain, were incorrectly placed and should have been assigned to *Pseudodoniella*.

A comparison of *Parabryocoropsis* with *Pseudodoniella* reveals that the former differs from the latter as follows: less elongate, being only about twice as long as wide; much shorter scutellum (shorter than its width at base), which is not visibly emarginate apically; abdominal connexiva more prominent; antennae shorter and thicker; cuneus much shorter in relation to width at base; posterior tibiae straight, thicker and feebly nodular, whereas in *Pseudodoniella* they are distinctly curved and minutely tuberculate.

With the transfer of *cheesmanae* and *duni* to *Pseudodoniella* this genus now contains five species all of which are associated with cacao and which may be distinguished by the following key.

Key to species of *Pseudodoniella*.

1. Scutellum black, ovoid in dorsal view 2
Scutellum not entirely black, trapeziform in dorsal view *pacifica*
China & Carvalho
2. Apex of scutellum in profile somewhat narrowly rounded 4
Apex of scutellum in profile broadly rounded 3
3. Head with elevation at base of tylus broadly and shallowly excised *duni*
(China & Carvalho)
Head with elevation at base of tylus narrowly excised *laensis*, **sp.n.**
4. Apex of scutellum rounded in posterior view *szentivanyi*, **sp.n.**
Apex of scutellum excised in posterior view *cheesmanae*
(China & Carvalho)

***Pseudodoniella laensis*, sp.n.** (fig. 1, d, e, f)

Colour: basal segment of antenna yellow, remaining segments brown; segment 2 with a faint yellowish suffusion basally. Head and thorax, except scutellum, yellow, the head pale. Posterior lobe of pronotum with a posterior irregular black spot of variable extent; scutellum black. Corium yellow; cuneus piceous; membrane infumate. Abdomen ventrally whitish yellow. Legs pale yellow, posterior femora suffused with piceous basally.

1 ♂ (holotype), 1 ♂ (paratype), Lae, 13.v.1951 (*G. S. Dun*); 6 ♂, 2 ♀ (paratypes), same locality, vi.1951 (*J. Hughes*) (B.M. 1951-390).

***Pseudodoniella szentivanyi*, sp.n.** (fig. 1, a, b, c)

Colour: basal segment of antenna yellow, remaining segments dark brown; segment 2 faintly suffused with yellow basally. Head and thorax, except scutellum, yellow. Posterior lobe of pronotum with a suffused brown spot

medially; scutellum black. Corium yellow; cuneus reddish yellow with apical margin piceous; membrane infumate. Abdomen whitish, connexiva narrowly piceous laterally; segments 5-7 suffused with piceous. Anterior legs yellowish,

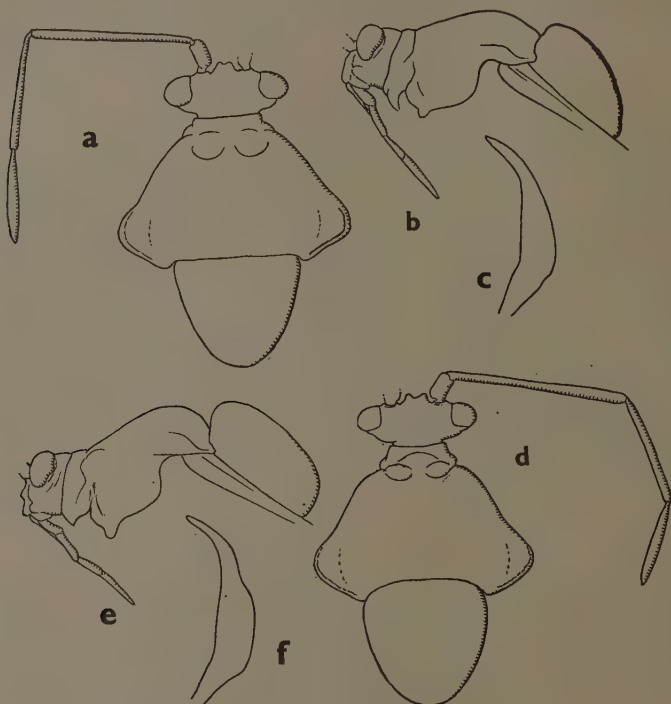


Fig. 1.—*Pseudodoniella szentivanyi*, sp.n. a, head, pronotum and scutellum (dorsal view); b, the same (lateral view); c, right harpago. *Pseudodoniella laensis*, sp.n. d, head, pronotum and scutellum (dorsal view); e, the same (lateral view); f, right harpago.

femora suffused with piceous; median and posterior legs with femora piceous, tibiae pale yellow with basal half piceous. The colour of the pronotum and corium varies to some extent. The former may be entirely yellow or almost entirely suffused with piceous or brown and the latter suffused with brown.

1 ♂ (holotype), 5 ♂, 15 ♀ (paratypes), Papua, Popondetta, 8-9.xii.1955 (J. J. H. Szent-Ivany).

The holotypes and paratypes are in the British Museum (Nat. Hist.), London.

Reference.

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THE SIMULIIDAE (DIPTERA) OF NORTHERN NIGERIA.

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The present paper * gives a general account of the SIMULIIDAE (Diptera) of Northern Nigeria, with particular reference to the distribution of the species, their breeding preferences, and the factors that appear to influence distribution.

Northern Nigeria has an area of 281,000 square miles and forms three-quarters of the total area of the Federation of Nigeria. The country, lying between 7-14° North and 3-14° East, is mainly between 1,000 and 2,500 feet above sea-level. although the central Jos (Bauchi) Plateau attains a height of 4,000 to 5,000 feet, and the Mambila Plateau of southern Adamawa Province reaches 6,000 feet and more. The country is well drained, most of the rivers rising on the Jos Plateau and flowing to the Niger or Benue Rivers. The valleys of the Niger and Benue are low-lying, and have an altitude of only 130 to 800 feet. In the far north, north of the 11° or 12° parallels, the rivers are sandy, shallow, and dried-up for several months of the year; south of this latitude some rivers may dry completely in the dry season, but most just manage to flow throughout the year. The rivers draining south-westwards from the Jos Plateau to the Niger, and those draining north-westwards from the southern Adamawa Hills to the Benue, are often rocky-bedded and swiftly flowing, particularly where they run off the escarpments into the Niger-Benue plain.

The seasons in Northern Nigeria are well defined, the rains lasting as a rule from May to October, and the dry season from November to April. In the far north the dry season lasts seven months, and the rains are only from May to September. In the southernmost areas the dry season is only of three months' duration. Rainfall over most of the country lies between 40 and 60 in. per annum, but in the extreme north does not exceed 15 to 25 in.; on the southern border it may reach 70 or 80 in.

Hitherto the SIMULIIDAE of Northern Nigeria have been extremely neglected, largely one supposes because the existence of onchocerciasis or "river blindness", transmitted by *Simulium damnosum* Theo., was until recently almost unrecognised in Nigeria. Very few records are given in the literature of any species of SIMULIIDAE from the north of Nigeria. The earliest report was that of Simpson (1912) who collected an unidentified man-biting *Simulium* in four localities, at Massamabu and Tegna in what is now Niger Province, at Otono on the Niger River above Bussa, and at Lokoja. *S. damnosum* is the only habitual man-biter in Nigeria, and has now been found at the four localities mentioned, so it seems fairly certain that Simpson's records refer to this species. De Meillon (1930) recorded *S. griseicollis* Becker from Northern Nigeria, but without any indication of the locality, though this is thought to be Ibi on the Benue River, for the British Museum collection contains three examples of this species collected from this locality by Dr. J. McF. Pollard in 1911. Recently, Freeman & De Meillon (1953) have recorded *S. bovis* De Meillon, *S. vorax* Pomeroy, and *S. medusaeformis* *hargreavesi* Gibbins from Nigeria, these records being based on collections made in the Northern Region. The author (Crosskey, 1956) has recently discussed the distribution of *S. damnosum* in Northern Nigeria, and this species is not considered in the present paper except to give a general account of its breeding habitat.

Between 1952 and 1955 an extensive survey for the breeding areas of Simuliids

* See p. 74.

was carried out in Northern Nigeria; this was designed to provide information on the distribution of onchocerciasis and of its vector, *S. damnosum*, but in addition the survey has incidentally shown the existence of a number of other species of SIMULIIDAE, many of which were previously unknown from Northern Nigeria. The survey covered nearly a quarter of a million square miles, some of it very remote country; no new species of SIMULIIDAE was discovered, and it was found that most of the Simuliids occurring in Northern Nigeria are ones of very general distribution in Africa.

The occurrence of all species was determined by the collection of the pupal stage. Adults of five species were caught wild, *S. damnosum*, *S. bovis*, *S. ruficornis* Macq., *S. griseicollis*, and a species of the *S. alcocki* group; only *S. damnosum* is a voracious feeder on man, although *S. bovis* can be a troublesome man-biter in very localised areas. *S. griseicollis* was found not to bite man in Northern Nigeria. Occasional adults of *S. damnosum*, *S. ruficornis* and a species of the *S. alcocki* group were caught at a light, about two hours after sunset.

List of Species.

Fourteen species of SIMULIIDAE have been found in Northern Nigeria. These are listed below, the species arrangement following that given by Freeman & De Meillon (1953).

Group I.	Group V.
<i>Simulium alcocki</i> Pomeroy 1922, type form <i>Simulium schoutedeni</i> Wanson 1947 <i>Simulium kenya</i> De Meillon 1940	<i>Simulium griseicollis</i> Becker 1903, type form; and form <i>tridens</i> Freeman & De Meillon 1953
Group II.	Group VII.
<i>Simulium cervicornutum</i> Pomeroy 1920, type form <i>Simulium unicornutum</i> Pomeroy 1920, type form	<i>Simulium medusaeforme</i> form <i>hargreavesi</i> Gibbins 1934 <i>Simulium vorax</i> Pomeroy 1922, type form <i>Simulium colas-belcouri</i> Grenier & Ovazza 1951 <i>Simulium bovis</i> De Meillon 1930 <i>Simulium damnosum</i> Theobald 1903.
Group III.	
<i>Simulium ruficornis</i> Macquart 1838	
Group IV.	
<i>Simulium hirsutum</i> Pomeroy 1922, type form <i>Simulium adersi</i> Pomeroy 1922	

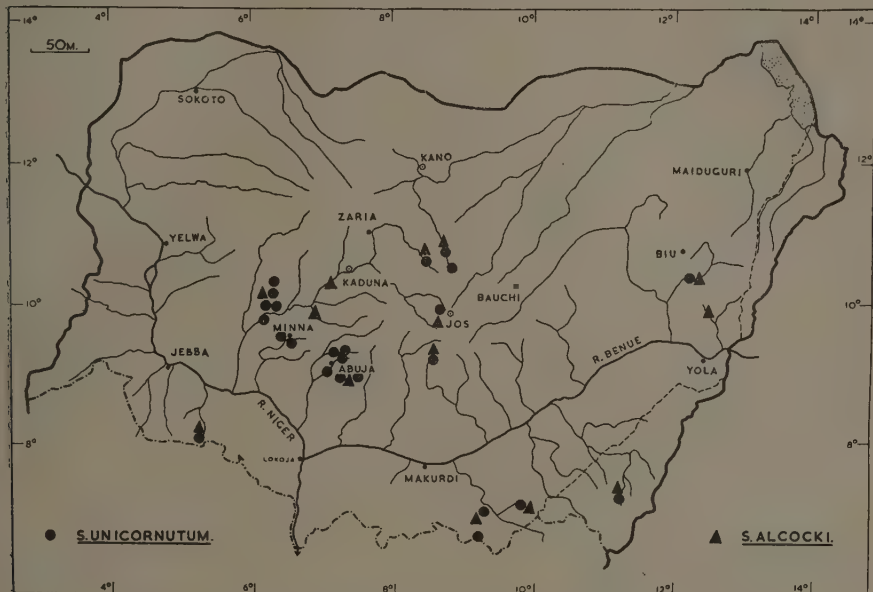
No species of Group VI, the group of *S. dentulosum* Roubaud, has been found. The species in Groups I-IV, and *S. colas-belcouri*, are recorded for the first time from Northern Nigeria. Some species and forms not known in the north are known from Southern Nigeria or the British Cameroons, or both, viz., *S. dentulosum*, *S. aureosimile* Pomeroy, *S. unicornutum* form *palmeri* Pomeroy, *S. alcocki* forms *occidentale* Freeman & De Meillon and *coalitum* Pomeroy, and *S. medusaeforme* Pomeroy type form (records in Pomeroy, 1920 & 1922, and in Freeman & De Meillon, 1953).

The records of *S. schoutedeni* and *S. hirsutum* provide connecting links between the previously recorded areas of these species (see Freeman & De Meillon, 1953), while the finding of *S. kenya* in Northern Nigeria is of interest in providing the first record of this species from West Africa.

It is worth noting that both the type form and form *tridens* of *S. griseicollis* occur in Northern Nigeria; the type form is by far the commoner, and has been found at intervals along some 350 miles of the Niger River. On the other hand, only a single pupa of form *tridens* has been found, this in the Kaduna River where the type form does not occur. Freeman & De Meillon (1953) have suggested that *tridens* is the usual form of *S. griseicollis* in Nigeria, but the observations mentioned above do not lend support to this view.

Distribution.

A complete list of locality records has been deposited in the archives of the British Museum (Natural History), and is available to any worker interested. The distribution maps of the various species (maps 1-5) are based on these records.



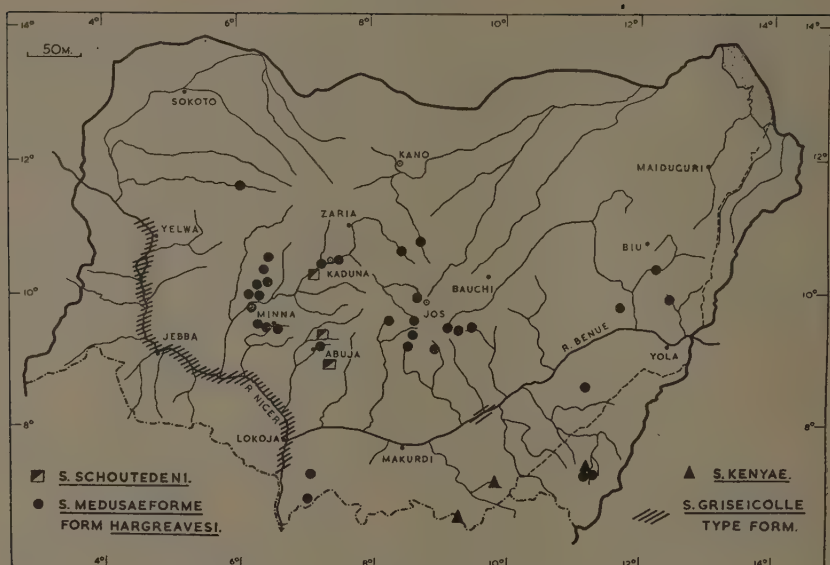
Map 1.—Showing the distribution of *Simulium unicornutum* and *S. alcocki*.

Simulium alcocki (map 1), *S. cervicornutum* (map 3), *S. unicornutum* (map 1), *S. ruficorne* (map 4), *S. adersi* (map 5), *S. medusaeforme* (map 2) and *S. damnosum* (maps given in Crosskey, 1956) are of fairly general occurrence wherever suitable rivers exist. Other species are more restricted, and *S. griseicollae* type form (map 2) is known only from the Niger River and *S. griseicollae* form *tridens* (map 5) has been found only in the Kaduna River near Zungeru, Niger Province. *S. hirsutum* (map 4) and *S. colas-belcouri* (map 3) have been found only in the Obudu Hills on the border of Tiv Division (Benue Province), while *S. kenya* (map 2) is also very localised, occurring only in the same locality and in the hills of southern Adamawa Province in the Cameroons Trusteeship Territory. *S. bovis* (map 4) appears to be confined to the central upland areas of the country, the Jos Plateau and its westward extensions; *S. vorax* (map 3) has been found only in the Assob River on the Jos Plateau at an altitude of 3,600 to 3,800 feet. *S. schoutedeni* (map 2) seems also to be a very localised species.

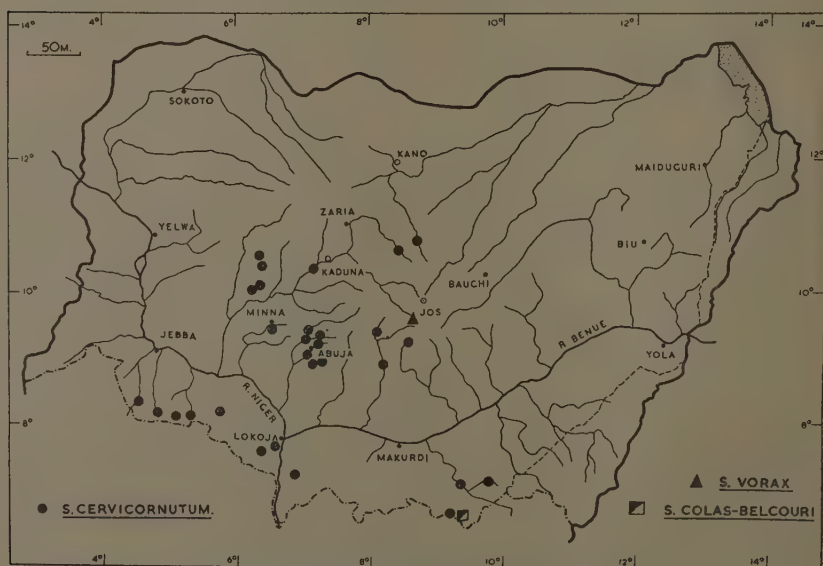
S. adersi occurs further north than the other species, and extends into the arid country north of 12° N.

S. alcocki and *S. unicornutum* are interesting in showing an almost identical pattern of distribution (map 1), and the immature stages of these species are almost invariably found associated together in the same streams.

Five main Simuliid areas may be distinguished in Northern Nigeria, and these are shown on map 6. The country in each of these areas is hilly, often extremely so, and has numerous rocky, swiftly flowing rivers which provide ideal conditions



Map 2.—Showing the distribution of *Simulium schoutedeni*, *S. kenya*, *S. medusaeforme* form *hargreavesi* and *S. griseicollis* type form.



Map 3.—Showing the distribution of *Simulium cervicornutum*, *S. vorax* and *S. colas-belcouri*.

for most species of Simuliids. Comparison of map 6 and maps 1-5 shows which species occur in each of the five main Simuliid areas.

Ecological Notes on the Species.

The Simuliid survey was based on the collection of the pupal stages of each species, and some information has been obtained on their breeding preferences. This is summarised in Tables I and II which show the type of river preferred for breeding, the altitudes at which breeding occurs, the normal substratum selected for attachment of the immature stages, and the association of each species with other species. A negative sign in the Tables indicates that the condition concerned is not a usually preferred one, not that the species is never found under this condition; for instance breeding of *S. damnosum* very occasionally occurs in streams, while pupae of *S. alcocki* have been found in large rocky rivers, but such conditions are unusual for these species and a negative sign is entered in the Tables.

Pupae of *S. alcocki*, *S. unicornutum*, *S. adersi*, *S. medusaeforme* and *S. damnosum* have been collected in all months of the year; those of *S. damnosum* are most abundant from May to October, *S. medusaeforme* from June to September, *S. unicornutum* in September, and *S. adersi* in the northern part of its range from January to March. *S. adersi* is unusual therefore in being most abundant in the late dry season when conditions are very severe, although this habit is also found in *S. ruficorne*, pupae of which have only been found from January to May. Pupae of *S. cervicornutum* are found in the late rains, through to the late dry season, i.e., from September to March, and appear to be absent during the early and mid rains. Pupae of *S. bovis* have been collected only in September and October, although rivers in which the species breeds have been surveyed in all months of the year and adult flies have been caught from June to October. Pupae of the remaining species have been found only in those months in which the survey visited the localised areas in which they occur: *S. schoutedeni* in December and April, *S. kenyae* in January and February, *S. hirsutum* in January, *S. griseicollae* type form in December to May (presumed to occur throughout the year), *S. griseicollae* form *tridens* in March, *S. vorax* in March, *S. colas-belcouri* in January and March.

S. griseicollae form *tridens* has been omitted from Table II, as only a single pupa of this form has been found. This was attached to a dead leaf in a large rocky river. Only the common or usual associations with other species are given in the Table; pupae of *S. alcocki* have sometimes been found on the same holds as pupae of *S. adersi* or *S. damnosum*, but such unusual associations are not tabulated as they apply only to occasional, isolated pupae. Pupae are regarded as clustered if they occur in large numbers immediately adjacent to one another, but not when they occur (as in the case of *S. cervicornutum* and *S. bovis*) in numbers but lie spaced out along the leaf-blade or other chosen hold.

The following supplementary notes on the various species may be given.

S. alcocki.

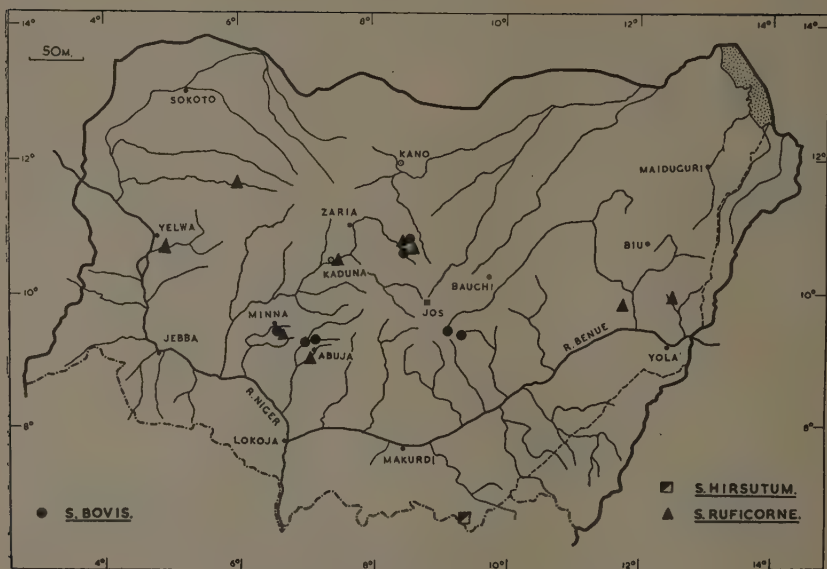
The pupal respiratory filaments usually number seven, but are sometimes eight, and pupae with seven on one side and eight on the other have been found.

S. schoutedeni.

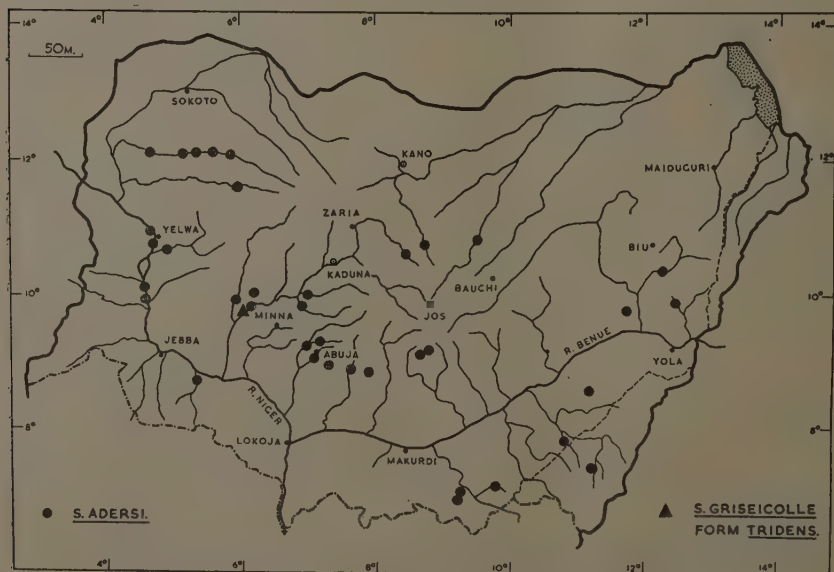
The pupae of this species are often difficult to distinguish from those of *S. alcocki*; they differ in being smaller, and having a long common stalk to the 8-filamented respiratory organ.

S. kenyae.

Although apparently a very localised species, pupae are abundant in those streams where the species does occur.



Map 4.—Showing the distribution of *Simulium bovis*, *S. hirsutum* and *S. ruficorne*.



Map 5.—Showing the distribution of *Simulium adersi* and *S. griseicollae* form *tridens*.

S. cervicornutum.

This species is very catholic in the type of breeding river, occurring as readily in fast-flowing rocky rivers as in slow placid streams. In material from Northern Nigeria, the pupal respiratory organ is always nine-branched; from most localities the branches lie in more or less the same plane, but in pupae from the Obudu Hills the most ventral branch lies in a plane at right-angles to the general plane of the respiratory organ, so that in side view the organ appears at first to be only 8-branched.

S. unicornutum.

Minor variations occur in the relative lengths of the two arms of the respiratory organ of this species; in pupae from the south-eastern parts of Northern Nigeria the point of attachment of the respiratory organ to the thorax is nearer the centre of the organ than in specimens from other parts of the country, where the horizontal arm is usually about twice as long as the upright arm.

S. ruficorne.

Pupae of this species are found only in streams in which scarcely any flow can be detected, and sometimes even in pools which are completely stagnant. Breeding does not occur in company with other species, as these normally cannot tolerate the conditions favoured by *S. ruficorne*. In Nigerian pupae the median filament of the respiratory organ divides very near the base, the two resulting median filaments are very nearly as long as, but narrower than, the posterior filament, and the anterior filament is thicker and longer than the posterior.

S. adersi.

This species is unusual in that it tolerates the severe dry-season conditions of the far north, breeding in the great sandy-bedded rivers of that area, providing these still just manage to flow during the desiccating hot season. In these rivers almost no holds for the immature stages of Simuliids occur, and the beds of the rivers are of shifting sand; but wherever a tuft of grass or a guinea-corn stalk is found in these rivers it will invariably bear larvae and pupae of *S. adersi*. The species also breeds readily in other areas of the country, but in the more central areas seems to prefer the presence of rapids to unbroken water. Except for *S. damnosum*, it is the only species that has been found breeding north of 12°N. In the far north, and in the Niger River, pupae are most prevalent in the mid dry season to late dry season, but in central and southern parts of its range are commonest in the rains.

S. griseicollae.

Immature stages of the type form have been found only in the Niger River. The Niger flows swiftly, and along several stretches is studded with islands, small islets, or floating masses of vegetation. Between the islands the water-channels are often very deep; where the water breaks round the upstream ends of the grassy islets the flow becomes very swift, and the pupae of *S. griseicollae* type form are found abundantly on the grasses and sedges dipping into the river. Occasional pupae are found attached to sticks, corn stalks and bamboo stems that have become lodged in the river. Between Jebba and Yelwa the Niger is rocky at intervals, particularly at the Bussa Rapids, and *S. griseicollae* is found breeding in these areas, as well as in places of smooth water.

S. medusaeforme.

Rock-surfaces, even in waterfalls, are sometimes favoured for attachment, and the larvae and pupae are occasionally present in such immense numbers as to give the rock-surfaces a blackened appearance. On grass stems and leaves they are

TABLE I.

Showing the preferred breeding habitats, and the altitudes at which breeding occurs, of the SIMULIDAE of Northern Nigeria.

Species	Breeding mainly in :—						Known breeding altitudes (ft. above sea-level)
	Slow muddy streams	Swift rocky streams	Small to medium rivers		Large rivers		
			Sandy or muddy	Rocky	Slow, sandy or muddy	Swift, rocky	
<i>S. alcocki</i> ..	++	—	+	—	—	—	1000-4000
<i>S. schoutedeni</i> ..	++	—	—	—	—	—	1400, 2000
<i>S. kengae</i> ..	—	+	—	—	—	—	1500-2000
<i>S. cervicornutum</i> ..	+	+	+	+	—	+	300-3000
<i>S. unicornutum</i> ..	++	+	—	—	—	—	mainly 1000-3000
<i>S. ruficornis</i> ..	+	—	—	—	—	—	900-2600
<i>S. hirsutum</i> ..	—	+	—	—	—	—	1500
<i>S. adersi</i> ..	—	—	+	+	++	+	600-4000
<i>S. griseicollis</i> type form	—	—	—	—	+	+	150-800 (Niger River)
<i>S. griseicollis</i> form <i>tridens</i>	—	—	—	—	—	+	850 (Kaduna River only)
<i>S. medusaeforme</i> ..	—	+	—	++	—	—	800-4500
<i>S. vorax</i> ..	—	+	—	—	—	—	3600-3800 (Assob River only)
<i>S. colas-belcourii</i> ..	—	+	—	—	—	—	1800 (Obudu Hills only)
<i>S. bovis</i> ..	—	—	—	+	—	—	300-4200
<i>S. damnosum</i> ..	—	—	—	+	—	+	150-4000

TABLE II.

Showing the preferred substratum for pupal attachment of the SIMULIIDAE of Northern Nigeria and the breeding association with other species.

Species	Pupae		Preferred pupal attachment				Species sometimes associated with :—
	Clustered	Not clustered	Trailing grasses	Sticks	Solid rock	Loose stones or boulders	Dead leaves
<i>S. alcocki</i>	—	+	—	—	—	+	++
<i>S. schoutedeni</i> ..	—	+	—	—	—	—	+
<i>S. kenyae</i>	+	+	++	—	—	+	+
<i>S. cervicornutum</i> ..	—	+	+	+	—	+	++
<i>S. unicornutum</i> ..	—	+	+	+	—	+	++
<i>S. ruficornae</i> ..	++	+	+	+	+	+	+
<i>S. hirsutum</i> ..	—	+	+	—	—	—	—
<i>S. adersi</i>	+	+	+	+	—	+	—
<i>S. griseicollae</i> type form	+	+	++	+	—	—	—
<i>S. medusaeforme</i> ..	++	+	++	+	+	+	—
<i>S. vorax</i>	—	+	+	—	—	—	—
<i>S. colas-belcourii</i> ..	+	—	+	—	—	—	—
<i>S. bovis</i>	—	+	+	—	—	—	—
<i>S. damnosum</i> ..	++	+	++	+	—	+	+
							<i>S. unicornutum, S. kenyae, S. cervicornutum, S. hirsutum</i>
							—
							<i>S. alcocki, S. hirsutum</i>
							<i>S. alcocki, S. damnosum, S. bovis</i>
							<i>S. alcocki, S. medusaeforme, S. damnosum</i>
							—
							<i>S. alcocki, S. kenyae</i>
							<i>S. damnosum</i>
							<i>S. damnosum</i>
							<i>S. unicornutum, S. damnosum, S. vorax</i>
							<i>S. medusaeforme</i>
							—
							<i>S. cervicornutum, S. damnosum</i>
							<i>S. cervicornutum, S. unicornutum, S. adersi, S. griseicollae, S. medusaeforme, S. bovis</i>

also usually very thickly massed after the manner of *S. damnosum*, and at first glance are easily confused with this species. In the pupal respiratory organ (form *hargreavesi*) the six primary filaments are long and the secondaries likewise, material from Northern Nigeria being similar to that from the Sudan (as figured by Freeman & De Meillon, 1953, p. 182). *S. medusaeforme* thrives in the brisk, stony, mountain streams of the Jos Plateau, at over 4,000 feet.

S. vorax.

This species is known only from the Assob River, a swift, tumbling, mountain stream on the Jos Plateau.

S. colas-belcouri.

In January 1955 the author collected some pupae of a doubtful species of *Simulium* from a fast-flowing stream in the Obudu Hills of Ogoja Province, on the border of Tiv Division, Benue Province, *i.e.*, the border of Northern and Eastern Nigeria. These pupae clearly belonged to a species of the *S. medusaeforme* group, but the respiratory organ showed differences from *S. vorax* and from typical *S. colas-belcouri*, although these seemed to be the nearest species. Some material including a slide of the male genitalia from a mature pupa was sent to Mr. P. Freeman, British Museum (Natural History), who very kindly compared it with material in the B. M. collection. The pupal respiratory organ showed a similar arrangement of the filaments to that in *S. taylora* Gibbins, but the filaments were much stouter, and a short stout filament was present on the posterior basal arm instead of on the anterior basal arm as in typical *S. taylora*; the male genitalia agreed with those of *S. colas-belcouri*, but were quite different from *S. taylora*. The pupae are regarded as belonging therefore to *S. colas-belcouri*, although the form of the respiratory organ is very much more slender, especially with regard to the basal arms, than in other material of this species from the Sudan and French Middle Congo. A description of the pupal respiratory organ is given below, and the organ is illustrated in the accompanying fig. 1; apart from the respiratory

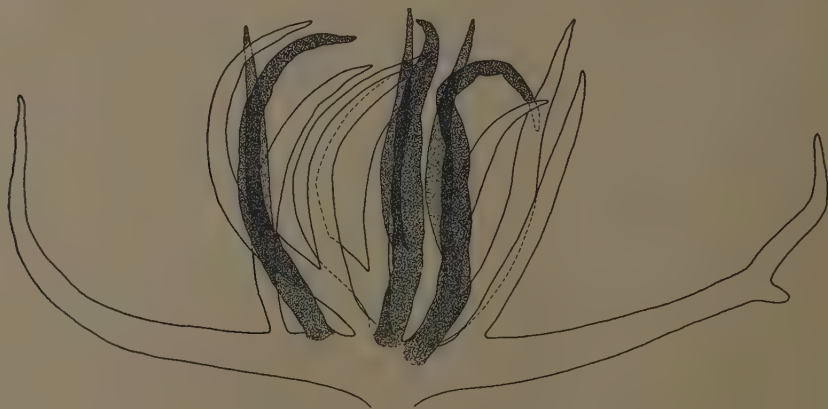


Fig. 1.—Outer view of left respiratory organ of pupa of *Simulium colas-belcouri* from the Obudu Hills.

filaments, the Nigerian pupae agree with pupae from elsewhere. *Respiratory organ*: with two relatively long, narrow, basal arms lying along the mouth of the cocoon and curving towards the corresponding filaments of the opposite side.

From the basal arms arise medially a total of 14 tubular digitate filaments, in two series. The outer series consists of three more or less upright thick-walled filaments, each with a secondary filament on the inner side, making a total of six filaments. The second series comprises eight thin-walled tubular arms; one arises from the base of the anterior basal arm, one from the base of the posterior basal arm, and the remaining six arise in pairs with a short common stem between these two. The walls of the outer series of six filaments are thicker, darker and more wrinkled than the walls of the inner series of filaments; the thick outer filaments are shaded in the illustration. The walls of the inner filaments are very thin and transparent. All the filaments curve inwards towards their fellows of the opposite side, and together rather suggest the appearance of a bunch of bananas. In addition to curving inwards, the filaments mostly curve backwards towards the posterior basal arm. The posterior basal arm terminates in a blunt stump, just anterior to which arises a short secondary filament. There is little distinction between primary and secondary filaments, as all are digitate and not truly filamentous. The inner series of filaments does not tend to lie against the thorax as in *S. natalense* De Meillon, and to some extent in *S. damnosum*. Under a hand-lens the respiratory organ appears whitish, and the pupae could be confused with those of *S. damnosum*.

S. damnosum.

This species breeds in rivers of very variable size, but not normally in streams, although one or two pupae may be found in small tributaries at the height of the rains. It almost always requires the presence of rapids and broken, well-aerated water, although occasional immature stages may be found in smooth, unbroken water away from the main breeding grounds. In Northern Nigeria, the confinement of the breeding of *S. damnosum* to the main rivers is most striking, and may greatly simplify control measures. In one focus, the Galma River near Kudaru in Zaria Province, surveys in tributaries for the immature stages of *S. damnosum* were made during each week of the wet season of 1953; although nine tributaries were surveyed, and five of these were rocky, fast-flowing and apparently suitable for the breeding of *S. damnosum*, not a single larva or pupa was found in any of the tributaries. In other foci, a very few pupae have sometimes been found in streams; some were collected in the Suka stream in Minna town in September 1954, although the permanent breeding ground is in the Chanchaga River, seven miles south of Minna. In general, however, watercourses under about 40 feet in breadth are not utilised for breeding, although these may be of a rocky nature, with broken water and abundant submerged grass and other vegetation. *S. damnosum* does not breed in major waterfalls, for, in general, suitable grass to provide holds for the early stages does not occur in such localities; in places where rapids exist, perhaps above or below waterfalls, but the fall is not steep, breeding may be found abundantly, especially if tufts of grass grow in the river bed. The presence of a small tree, *Salix Ledermannii*, known in the local Hausa language as "baruwana", growing on banks in the river bed, seems to be frequently associated with the breeding of *S. damnosum* between 9° and 12° North. In the few foci where *S. damnosum* exists north of 11° the rivers are very large and sandy, and only here and there are there very localised places in the river beds that are stony. There is almost no vegetation in these rivers except for tufts of a short bright green unidentified sedge (Hausa: "aya-aya") which grows among pebbles where the bed is stony; the immature stages of *S. damnosum* are sometimes found on the leaf-blades of this sedge in company with those of *S. adersi*. *S. damnosum* is quite often found breeding on tufts of grass growing among boulders that have been put down in the river bed to form a drift for the passage of motor vehicles; in such cases the drift usually causes an artificial speeding-up of river flow, and conditions favouring *S. damnosum* result.

The presence of trailing submerged grass is almost indispensable for breeding of *S. damnosum*, for the larvae and pupae are almost always found attached to grasses, and only rarely to the leaves of trees dipping into a river, to dead leaves or rocks and stones. Occasionally the larvae and pupae may be found attached to sticks, branches, corn stalks, palm fronds and such like that have become lodged among rocks, and over which the water breaks with some force. In the dry season a few pupae attach themselves to stones and boulders, but grass is by far the most favoured hold at all times of the year. Wherever the conditions described above occur in Northern Nigeria *S. damnosum* seems to be found; any river of reasonable size (about 40 ft. across and upwards), with a partially rocky bed providing broken and spilling water, where grass grows plentifully, and the flow continues throughout the year, is almost certain to support breeding of *S. damnosum*. In Northern Nigeria, the species does not breed to any extent in rivers which dry up in the dry season; dry-season survival is maintained as a rule by continuous breeding on a reduced scale (Crosskey, 1955).

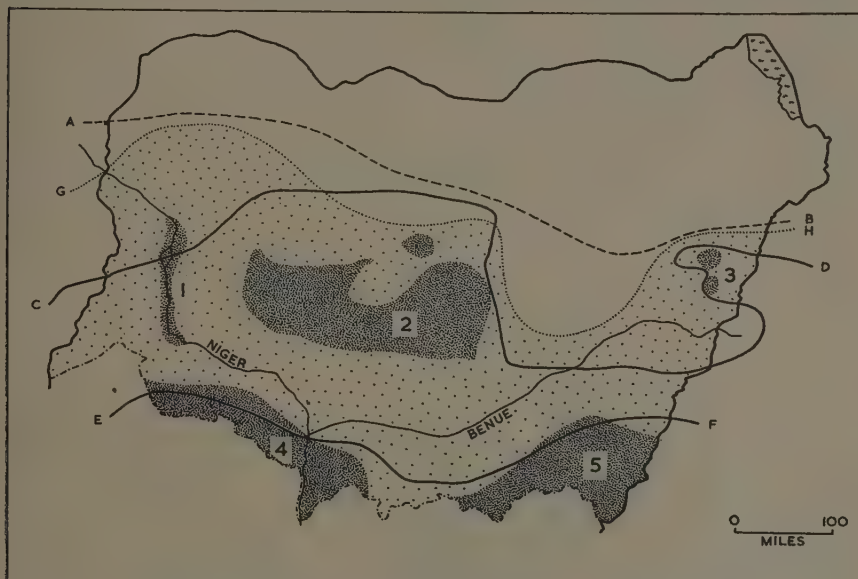
Discussion of Simuliid Distribution.

The distribution of SIMULIIDAE is undoubtedly governed by the localisation of suitable breeding grounds. The majority of species require a specialised habitat, usually swiftly flowing rivers with rocky beds providing broken well-aerated water, and the presence of partially submerged vegetation; in addition, perennially flowing water, at least in some of the rivers of any particular focus, appears to be indispensable for the survival of most species. The specialised conditions required by SIMULIIDAE are by no means universal in Northern Nigeria, and over many thousands of square miles of relatively flat country where the rivers are sandy or muddy, or the dry season very protracted, SIMULIIDAE either do not exist or are found only to a very minor extent.

The factors primarily determining the distribution of SIMULIIDAE are therefore markedly different from those which appear to be responsible for limiting the range of other blood-sucking insects, for example, the tsetse flies. The distribution of some species of tsetse depends essentially upon the climatic conditions existing in particular botanical associations—in the case of *Glossina tachinoides* Westw. on the temperatures and humidities experienced in the riverine fringing forest of the savannah-woodland areas. But in the case of SIMULIIDAE it seems improbable that climatic conditions, with the possible exception of rainfall, affect to any very great extent the distribution of the species, except under the hot desiccating conditions of the far north, north of latitude 11°. In these northerly areas, however, the river beds are almost never rocky, and fast broken water does not occur; the scarcity of SIMULIIDAE in the far north is much more certainly attributable in the first instance to this factor than to the climatic one. Again, suitable vegetation for providing larval and pupal holds is normally absent from these northern rivers, and this in turn may well be a factor limiting the distribution, acting in conjunction with the geological and climatic conditions.

Over most of Northern Nigeria it is unlikely that the amount of rainfall plays any direct part in deciding Simuliid breeding areas; SIMULIIDAE are as prevalent where the mean annual rainfall is over 80 in., as in other areas where it is no more than 45 in. But the number of dry-season months, that is to say, months with one inch of rainfall or less, evidently plays an important part in determining seasonal abundance, though it does not in itself prevent the occupation of any areas by SIMULIIDAE, except perhaps the semi-desert regions along the northern border of the country. In this connection the approximate boundary line between areas with seven dry-season months, and those with six or less, is shown by the line A-B on map 6. It will be seen that SIMULIIDAE do not occur in the area with a very prolonged seven-month dry season; in this area most rivers dry completely in the course of the dry season, or at any rate cease to flow and contract to an

occasional pool. The effect of the absence of rainfall is to create a long desiccating hot period during which the drying Harmattan wind may blow for two or three months. But as pointed out above, other conditions in the far north are also inimical to SIMULIIDAE; hence the absence of Simuliids from this area is probably attributable to all or some of the following factors in combination:— (i) the absence of rocky beds and broken water from the rivers; (ii) the absence of suitable



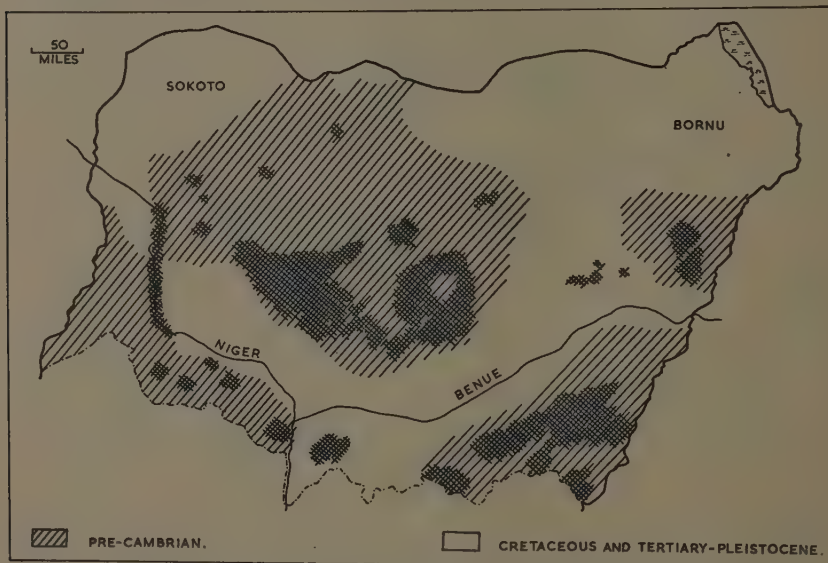
Map 6.—Showing the general distribution of SIMULIIDAE in Northern Nigeria; heavy shading represents the main breeding areas. 1. Niger River area; 2. Central area; 3. Northern Adamawa area; 4. Kabba-Ilorin area; 5. South-eastern area. A-B: line representing the approximate boundary between the area with a seven-month dry season and that with six dry-season months or less. C-D: southern limit of Sudan Savannah and northern limit of Guinea Savannah. E-F: southern limit of Guinea Savannah and northern limit of Derived Savannah. G-H: approximate northern limit of perennial breeding of SIMULIIDAE.

holds in the rivers; (iii) the low rainfall of short duration, resulting in a very prolonged dry season; (iv) the hot desiccating conditions with extremely low relative humidity.

The type of botanical association, even the marked separation of forest from savannah, seems to have no significance as far as SIMULIIDAE are concerned. Usually the species of the northern savannah areas of Nigeria are equally in evidence in the forested regions of southern Nigeria and the Cameroons. True high forest areas no longer exist in Northern Nigeria, although in earlier times the forest is believed to have extended as far north as the line E-F marked on map 6. Northwards of this line is original savannah country, the area between the lines E-F and C-D (see map 6) being termed Guinea Savannah, and that north of the line C-D Sudan Savannah. The latter differs from the Guinea Savannah in having a growth of shorter, sparser trees, often thorny species, and in the shorter grass cover. The area south of the line E-F is usually termed Derived Savannah, to indicate that it has been derived from the true forest by the gradual encroachment of the Guinea Savannah. Within the Derived Savannah, extensive relict patches

of earlier forest still exist, while in the Guinea Savannah, fringing forest occurs along the banks of rivers and streams and occasional small forest "kurmis" are found in places (*e.g.*, Abuja Division) where local rainfall conditions encourage a more luxuriant growth. The forests of the Guinea Savannah are, however, extensions from the main forest block in southern Nigeria, and not remnants of an area formerly completely forested (Rosevear, 1953). The five main Simuliid areas (see map 6) lie within the Guinea or Derived Savannah, and only a limited number of breeding foci exist outside of these areas in the Sudan Savannah. Nevertheless it is not to be supposed that the vegetation zones in any way determine the *Simulium* zones; the association is most probably coincidental, as the suitable breeding rivers occur only within the areas clothed with Guinea or Derived Savannah. The Sudan Savannah, from which SIMULIIDAE are largely absent, occurs in areas with low rainfall and prolonged dry season, mostly on Cretaceous or more recent deposits where rocky-bedded rivers are absent.

Geologically, about half the land surface of Northern Nigeria is composed of ancient pre-Cambrian rock, or "basement complex" (map 7). Over the remaining



Map 7.—Showing the geology of Northern Nigeria; the cross-hatching represents the area of the pre-Cambrian "basement complex". The unshaded area is composed of sedimentary deposits of Cretaceous or late-Tertiary to Pleistocene age. Heavy shading indicates the areas in which fast-flowing rocky-bedded rivers occur.

areas, the valleys of the Benue and lower Gongola Rivers, most of the Niger valley, and the Bornu and western Sokoto plains, the basement complex is overlain by sedimentary deposits laid down in Cretaceous or late-Tertiary to Pleistocene times. The basement complex is composed of resistant rocks, largely granites, and where these hard pre-Cambrian formations form the land surface (*i.e.*, over most of central Northern Nigeria, south-west of the Niger, and in Adamawa Province) the rivers are characteristically rocky-bedded, sometimes with picturesque rapids and waterfalls, and favourable conditions for SIMULIIDAE occur. On the other hand, those areas which were formerly submerged by the sea and now show

Cretaceous or Tertiary-Pleistocene sandstones and other sedimentary formations in general present smooth rivers with beds of sand or mud, very rarely with any outcrops of hard rock. Such rivers are normally devoid, or almost so, of Simuliid breeding, as for instance the rivers of the Benue, Gongola and Bornu plains.

The association of SIMULIIDAE with the areas of the basement complex and not with the areas of later sedimentary deposits is most striking, and is demonstrated by a comparison of maps 6 and 7. On map 7, the area of the basement complex is shown by the cross-hatching, and those areas which typically show rocky-bedded rivers are indicated by the heavily shaded patches. It will be noticed that the areas of rocky or stony rivers occur almost without exception in the pre-Cambrian basement complex; a comparison of these areas with the main breeding areas of SIMULIIDAE, shown on map 6, presents a well-marked correlation. This is well shown by the upper Niger River from Yelwa to Jebba; this stretch is often rocky with rapids which form the breeding grounds of Simuliids, but it is also the only stretch where rocky outcroppings of the basement complex occur. In some restricted areas of the basement complex the rocky beds of the river have been produced by the invasion of the old pre-Cambrian—either by younger granites (Jos Plateau) or by volcanic outpouring of lava (Biu Plateau). The latter case is well shown by several miles of the Hawal River, a breeding ground on the borders of Biu Division of Bornu and Adamawa Province, where the river bed is composed almost entirely of a most unusual fissured lava rock.

It may be noted that the altitude of the basement complex is from a few hundred to over 5,000 feet above sea-level, and that almost all the Simuliids of Northern Nigeria occur at various altitudes between these limits. Altitude appears to play no part in determining the breeding zones, except perhaps in the case of *Simulium vorax* which is known only from a localised area at 3,600 feet to 4,000 feet.

To summarise the discussion given above the following points may be made. (1) Simuliid distribution depends upon the distribution of suitable breeding rivers. (2) The distribution of such rivers depends upon the geology of the country, upon the area of the pre-Cambrian basement complex. (3) The overall picture of Simuliid distribution is probably not dependent upon vegetation zones, although most breeding grounds occur in the Guinea Savannah. (4) Altitude is apparently of no importance in the distribution of most species. (5) Except in the far north, climatic factors in general seem to have little direct influence upon SIMULIIDAE, except in so far as rainfall is responsible for the continuous flow of the breeding rivers, although microclimatic considerations probably play a part in any particular focus. (6) The northerly limit of SIMULIIDAE is fixed by the absence of suitable rivers, and perhaps by the long duration of the dry season.

Summary.

Fourteen species of SIMULIIDAE (of which one occurs in two forms) are recorded from Northern Nigeria. Localities for each species are given on distribution maps. Most of the species are of fairly general occurrence in the Ethiopian Region, although *Simulium kenyae* De Meillon is recorded for the first time from West Africa. No new species was found in an extensive survey of about 200,000 square miles.

Some notes are included on the breeding habits of the species, particularly the type of breeding site preferred, the altitudes at which breeding occurs, and the months in which immature stages have been found.

Some discussion is given of the distribution of SIMULIIDAE in Northern Nigeria and the factors which appear to influence it, partly illustrated by maps. The most important factor is thought to be the geology of the country, which determines the areas of rivers suitable for breeding. Other factors such as climate, altitude, or vegetation zones, seem to play little part, except in the far north.

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APPENDIX.

Since this paper was submitted for publication, *S. ruficorne* has been taken in the arid country, north of 12° N., in which only *S. adersi* and *S. damnosum* were previously known to occur (see pp. 61 & 65). The distribution of *S. ruficorne* shown in map 4 is, therefore, incomplete. Further work has also shown that pupae of *S. ruficorne* and *S. bovis* are to be found in all months of the year, and that, in addition to the periods recorded, a few pupae of *S. cervicornutum* are present during the early and mid rains. Statements on p. 63 should be qualified accordingly.

Further observations suggest that modifications are necessary to the statement (pp. 70–71) that SIMULIIDAE do not occur in the area with a seven-month dry season. Some seasonal breeding does occur north of the line G–H on map 6.

A SURVEY OF THE BUILD-UP OF INFESTATION IN BAGGED COCOA BEANS IN STORE IN WESTERN NIGERIA.

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The aim of the present work has been to investigate the rate of build-up of insect populations in a stack of bagged cocoa beans, under normal field conditions, during the greater part of the period in which main-crop cocoa is stored before export.

The work was carried out on a stack of 2 tons of cocoa in 32 bags, situated in a typical cocoa store at Ibadan. The cocoa was stored from the time of grading in the middle of December 1953 until the end of April 1954 a period of 19 weeks.

During most of this time other cocoa was in the store, but it was only in February and March that the store was more than half filled. From the end of March several stacks of palm kernels were in the store, and little other cocoa was present.

Methods of Sampling.

The catches of insects by "até" strands, and the numbers of insects and damaged beans found by examining samples taken with a sampling spear, were both used as indices of infestation. "Até" strands consist of mid-ribs of palm leaves coated with a local bird-lime mixture (Golding, 1941).

Approximately 30 "até" strands were hung vertically round the stack of bags. At weekly intervals, the number of insects caught was recorded, and the strands replaced by a series of freshly made ones. Previous tests had shown that the catch by "até" strands did not vary greatly with age during the first week after being prepared.

Spear samples were taken every two weeks. On each occasion five samples, each of 30 beans, were taken from each bag. The aim was to take the largest number of beans which could be conveniently examined in the time available, and to distribute the sampling equally over the whole stack. Records were kept of each individual sample, in order to discover whether there was any noticeable variation of infestation in different parts of the stack.

The Build-up of Infestation.

The infestation proved too low for anything but an overall picture of infestation trends to emerge and there was no suggestion that there was any variation between different parts of the stack.

In Table I the catches on "até" strands are recorded as the mean catch per week. The number of strands on which these means were based varied from 28 to 32. The catches on individual strands vary considerably, and any attempt to express the variability of each week's mean would lead to a confusing picture. "Até" strand catches should only be used to estimate the general state of infestation at any one time, or trends of population changes over a period of at least several weeks.

The percentage of beans damaged by insects, and the percentage containing the two most serious pests, *Lasioderma serricorne* (F.) and *Ephestia cautella* (Wlk.) have been calculated as means of a series of five samples, each sample consisting of 30 beans from each of the 32 bags. Thus each mean is based on an

examination of 4,800 beans. Again, the errors of the means are considerable, in this case, because of the small numbers of insects present, and each figure expressed cannot be regarded individually but only as a part of the series.

The figure for the "percentage damaged by insects" at the start of the experiment was 0.11 (Table I) and this number represented beans damaged by *E. cautella*. As the experiment progressed, more and more beans were damaged,

TABLE I.

A summary of the numbers of insects and infested beans seen from week to week over a period of 19 weeks.

No. of weeks from grading	Month	Mean catch on one "até" strand during each week			Means of five samples, each of 960 beans		
		<i>Ephestia cautella</i>	<i>Lasioderma serricorne</i>	<i>Tribolium castaneum</i>	Percentage damaged by insects	Percentage containing <i>Ephestia cautella</i>	Percentage containing <i>Lasioderma serricorne</i>
1 } 2 }	Dec.	0.7 0.2	0.00 0.06	0.00 0.06	0.11	0.00	0.00
3 } 4 } 5 } 6 } 7 }		1.4 2.8 6.6 2.2 1.6	0.08 0.06 0.27 0.13 0.09	0.04 0.10 0.13 0.06			
8 } 9 } 10 } 11 }	Jan.	1.6 5.9 17.4 17.1	0.05 0.15 0.06 0.07	0.02 0.05 0.09 0.10	0.17	0.06	0.02
12 } 13 } 14 } 15 }		21.8 5.7	0.84 2.03	0.65 1.27			
16 } 17 } 18 } 19 }	Mar.	4.9 20.2 13.8 9.9	1.72 2.32 1.93 3.72	2.00 0.86 0.86 1.22	0.33	0.06	0.04
	Apr.				0.44	0.02	0.15
					0.85	0.02	0.27
					0.85	0.02	0.31

principally by *E. cautella* and *L. serricorne*, but also to a lesser degree by *Tribolium castaneum* (Hbst.) and *Laemophloeus* sp. This explains why it is that the percentage damaged towards the end of the period was so much in excess of the sum total of the percentages containing *E. cautella* and *L. serricorne*.

Not all the species counted have been presented in the Table. A few examples of *T. castaneum* and *Laemophloeus* sp. were found inside beans, but their numbers were too low to be suitable for tabular presentation, or to show any trends of infestation development. The "até" strands trapped some *Laemophloeus* sp., but it was considered that this insect, and *Ahasverus advena* (Waltl) which was also present, were too small in size for reliable counts to be made. A number of other insects trapped by "até", such as *Carpophilus* spp., *Necrobia rufipes* (Deg.) and *Araecerus fasciculatus* (Deg.) were not considered to be feeding on cocoa, since they were never encountered in sampling. Cotterell (1952) states that *A. fasciculatus* is becoming less frequent on cocoa owing to improved drying conditions. Both *Carpophilus* spp. and *N. rufipes* tended to occur on the strands only when palm kernels were present in the store.

Although the catches on the strands suggest that *E. cautella* is the most numerous insect on cocoa, the results of the bean sampling show that its actual population is small throughout the season, and there is no sign of a major build-up of population. This latter feature of infestation by *E. cautella* is shown in the "até" strand catches, for, from mid-February until the end of April there is no overall increase in catch. *Lasioderma serricorne* is clearly the most serious pest, its population increased steadily from an extremely low level, and after 19 weeks it had increased more than 1,500 per cent. An initial estimated population of less than 200 beetles in the 32 bags increased to one estimated to be some 6,400 beetles after 19 weeks. On cocoa, the average life-cycle of *L. serricorne* is about ten weeks under normal January to March conditions.

This increase then represents the breeding of about two generations; at the same rate of increase the number of beetles after three generations would be about 36,000. It would therefore seem that the longest time that cocoa can safely be stored in Nigeria is 20-25 weeks. *i.e.*, 2-2½ times the average development period of the beetle.

Total Numbers of Insects seen.

Table II shows the totals of the various species of insects seen on "até" strands and from spear samples. They are arranged in probable order of economic importance.

TABLE II.

The total numbers of various species seen during the survey.

Species	In samples of beans	Caught on "até" strands
<i>L. serricorne</i>	43	518
<i>E. cautella</i>	15	4,977
<i>T. castaneum</i>	10	324
<i>Laemophloeus</i> sp.	11	6
<i>A. fasciculatus</i>	0	45
<i>Carpophilus</i> spp.	0	120
<i>N. rufipes</i>	0	33
<i>Piezostethus</i> (?)	0	1
<i>Dermestes</i> sp.	0	1
<i>Ahasverus advena</i>	0	1
<i>Tenebroides mauritanicus</i> (L.)	0	1

It can be seen that the catches on strands hung round a stack of cocoa in no way reflect the proportions of the various species present in the cocoa.

Summary.

Two tons of cocoa, in 32 bags, stacked in a typical cocoa store in Ibadan, Nigeria, were sampled from December 1953 to the end of April 1954. The weekly catches on "até" strands hung round the stack suggested that *Ephestia cautella* (Wlk.) was the most numerous insect on cocoa, but spear samples of the beans, taken from the sacks every two weeks, showed that the actual population of this species in the beans was small throughout the season, with no sign of build-up. *Lasioderma serricorne* (F.), on the other hand, was shown to be the most serious pest, the population in the beans increasing steadily from an extremely low level. It is estimated that on the average it is not safe to store initially good cocoa for more than 25 weeks in the main crop season.

Acknowledgements.

Thanks are due to Messrs. John Holt, Ltd. for their co-operation in allowing the use of part of a warehouse. Thanks are also due to the Produce Inspection Service of the Department of Marketing and Exports at Ibadan.

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THE SPECIFIC STATUS OF *CALLOSOBRUCHUS MACULATUS*
(F.) AND *CALLOSOBRUCHUS ANALIS* (F.).

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and

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(PLATES III & IV.)

From the original descriptions and subsequent published literature on the two storage Bruchids, *Callosobruchus maculatus* (F.) and *Callosobruchus analis* (F.), it is impossible to decide whether or not these are distinct species. Herford (1935), following Dr. K. G. Blair, treats these two names as synonyms, as do Lepesme (1945) and Decelle (1951). Bridwell (1938), on the other hand, considers these species to be distinct, as did the late Mr. D. J. Atkinson of the Commonwealth Institute of Entomology. Zacher (1952) listed *C. analis* as a synonym of *C. maculatus* but later (Zacher, 1954) as separate species. Mukerji & Chatterjee (1951) in a paper on Indian Bruchids, describe the genitalia of *C. analis* but do not mention *C. maculatus*. Utida (1954) describes two forms or "phases" of *C. maculatus* in Japan.

The position is further complicated by doubts concerning the synonymy of *maculatus*. In particular, the opinion of Fähræus (in Schönherr, 1839) that *Bruchus quadrimaculatus* F. was a synonym of *maculatus* was not accepted by Pic (1913) in his catalogue, and subsequently some authors have followed one view and some the other.

The work described in this paper was started when one of the authors (G.A.B.) obtained living specimens of a Bruchid that was quite distinct from the relatively common *C. maculatus* and concluded that it was almost certainly *C. analis*. With living material of both species available, it was obviously desirable to publish adequate descriptions of them, with emphasis on the characters by which they could be separated. *C. maculatus* is very variable and an important reason for its confusion with *C. analis* is that many specimens of *C. maculatus* have the rufous ground colour of *C. analis*, whereas other specimens of *C. maculatus* have a black or dark brown ground colour. The original description of *maculatus* mentions testaceous antennae, whereas that of *quadrimaculatus* mentions black antennae; we are satisfied that these descriptions were based on forms of *C. maculatus* similar to those produced in cultures maintained at the Pest Infestation Laboratory.

Systematics.

Callosobruchus is used here as the generic name of the two species that are the subject of this paper. This name was proposed by Pic (1902) for a new sub-genus of *Bruchus*. Bridwell (1929) considered this sub-genus to be of generic status and designated *Bruchus scutellaris* F. (= *Curculio chinensis* L. 1758), as the type species.

Fabricius (1781) described *Bruchus analis* from the Oriental region and the type specimen is now deposited in the British Museum (Natural History). This type was examined by one of us (B.J.S.) in company with Mr. R. D. Pope of the Commonwealth Institute of Entomology, and was found to agree with the culture material upon which the descriptions of *Callosobruchus analis* in this paper are based.

The collection of *C. analis* in the British Museum (Natural History) includes specimens that had originally been named *Bruchus jekeli* Allib. and *B. glaber* Allib. by Pic. If Pic's identifications were accurate, these names must be considered synonyms of *analis*. The whereabouts of the types of these two species is unknown. Pic (1913) lists them as distinct species, but the original descriptions (Allibert, 1847) are consistent with the suggestion that they are synonyms of *analis*.

Fabricius (1775) described *maculatus* from America and placed it in the genus *Bruchus*. The collection of the University Zoological Museum, Copenhagen, possesses two specimens, which were almost certainly examined by Fabricius. They are labelled exactly as indicated in Fabricius' original description "Ins: Amer: V: Rohr. tws: S. & J. L." They are thought by the Museum to be the type material and were examined for us by Dr. S. G. Larsson. He decided that one specimen corresponded with material of *C. maculatus* bred at the Pest Infestation Laboratory, but was not convinced that the other was the same species. He very kindly sent these two specimens to the Pest Infestation Laboratory where they were examined by two of us (B.J.S. and R.W.H.). The first specimen corresponded in every respect with the culture material of *maculatus* used for the description given in this paper, and this individual from the von Rohr collection is therefore designated as the *lectotype* of *maculatus* F. The other specimen was found to be *C. analis*, a species not otherwise, to our knowledge, recorded from America.

Ten names are mentioned in the literature as synonyms of *maculatus*. Bridwell (1929) mentions six of these as names that had been applied to specimens of *maculatus*, but he omits them from a list supplied subsequently to Larson & Fisher (1938) and it is not recorded that he proved that they were synonyms; it must be assumed, therefore, that he failed to establish them as such. Two of the ten names are shown here to be synonyms of *chinensis* L., not of *maculatus*. Five names are definitely established here as synonyms of *maculatus* and opinions from the literature are cited. Notes on the ten names are given below.

1. *quadrifasciatus* Fabricius, 1792. This name was regarded as a synonym of *maculatus* by Schönherr (1839), Horn (1873), Bridwell (in Larson & Fisher, 1938), Hoffmann (1945) and Decelle (1951). Pic (1913) and Lepesme (1945) list *maculatus* and *quadrifasciatus* as distinct species. The type of *quadrifasciatus* is held at the University Zoological Museum, Copenhagen, and was examined for us by Dr. Larsson. He found that it was identical with our culture material of *maculatus* and with the specimen designated above as the *lectotype* of *maculatus*, except for the difference of antennal colour noted above. The name *quadrifasciatus* F. must therefore be accepted as a synonym of *maculatus* F.

2. *barbicornis* Fabricius, 1801. Although the original description clearly states that the antennae of this species are pectinate, it was listed as a variety of *quadrifasciatus* by Pic (1913) and as a synonym of *maculatus* by Hoffmann (1945). Bridwell (1929) apparently considered this name to be a synonym of *maculatus*. The type of *barbicornis* is also held at Copenhagen and has been examined for us by Dr. Larsson. He was convinced that this specimen was not *maculatus* but was identical with *scutellaris* F. (= *chinensis* L.), an opinion held by Schönherr (1839).

3. *bistriatus* Fabricius, 1801. This species was also listed as a variety of *quadrifasciatus* by Pic, and as a synonym of *maculatus* by Hoffmann. Again

Bridwell apparently disagreed. Schönherr (1833) listed *bistriatus* as a distinct species, but in 1839 gave the name as a synonym of *scutellaris* F. The type material is held at Copenhagen and was considered by Dr. Larsson to be *scutellaris* F. Dr. Larsson kindly sent to us the two specimens of *bistriatus* from the Fabrician private collection (Kiel-collection), and one of the two female specimens forming the type series of *scutellaris* F. All these specimens are conspecific with the type of *Curculio chinensis* L., in the collection of the Linnean Society of London, which has been examined by one of us (B.J.S.).

4. *chinensis* Thunberg, 1816. This name is given as a synonym of *maculatus* F. by Bridwell (in Larson & Fisher, 1938) whereas Pic (1913) lists it as a synonym of *chinensis* L., 1758. We (B.J.S. & R.W.H.) have examined the only specimen bearing the name *B. chinensis* in the Thunberg collection held by the Museum of the Zoologiska Institutionen, Uppsala. This specimen corresponds with Thunberg's description but it does not belong in *C. maculatus* (F.), or in *C. chinensis* (L.). It is, in fact, a member of the genus *Bruchidius*, but one previously unknown to the present authors. We feel therefore that *Bruchus chinensis* Thunberg is a valid species of *Bruchidius*. The name *chinensis* being preoccupied in that genus (Linnaeus, 1758), we propose the name *Bruchidius thunbergi* as the new name and new combination for *Bruchus chinensis* Thnb. (nec L.).

5. *longicornis* Thunberg, 1816, is given as a synonym of *maculatus* by Bridwell (in Larson & Fisher, 1938) but is listed as a separate species by Pic (1913). It is not represented in Thunberg's collections at Uppsala. However, it is clear from the original description, where the femora are described as "omnia inermia", that *longicornis* is not even a species of *Callosobruchus*, much less synonymous with *C. maculatus*. In our opinion, *longicornis* should be regarded as a valid species of *Bruchus* until further information becomes available.

6. *ornatus* Boheman, 1829, is listed as a variety of *maculatus* by Pic (1913). Examination of the type specimen shows it to be *maculatus* F., although Bridwell (1929) apparently was unable to establish this synonymy.

7. *litteratus* Schönherr, 1833, is given as a synonym of *maculatus* by Bridwell (in Larson & Fisher, 1938). It is listed as a synonym of *longicornis* Thnb. by Pic (1913). *B. litteratus* Schönh. (1833) was a new name unnecessarily proposed by Schönherr for *B. longicornis* Thnb. (see above), of which it is thus a synonym.

8. *vicinus* Gyllenhal, 1833 (in Schönherr, 1833). The type specimen is an example of *C. maculatus* (F.), with which *B. vicinus* Gylh. is thus synonymous.

9. *ambiguus* Gyllenhal, 1839 (in Schönherr, 1839). Bridwell (1929) includes this in a list of names "which have been supposed to fall into" the synonymy of *C. maculatus* (F.). He omits *ambiguus* from his list in Larson & Fisher (1938). The type specimen and a paratype are examples of *C. maculatus* (F.), with which *ambiguus* Gylh. is thus synonymous.

10. *sinuatus* Fähræus, 1839, was placed as a synonym of *maculatus* by Horn (1873) and as a variety by Pic (1913). Bridwell (1929) quotes the name in his earlier list of synonyms of *maculatus* (F.), but not in the list supplied to Larson & Fisher (1938). We (B.J.S. & R.W.H.) have examined the type specimens of *B. sinuatus* Fähr. and *B. sinuatus* var. β , Fhs. Both are examples of *C. maculatus* (F.) with which *B. sinuatus* Fhs. is thus synonymous.

TABLE OF SYNONYMY.

Callosobruchus maculatus (F.)*Bruchus maculatus* F., 1775*Bruchus 4-maculatus* F., 1792*Bruchus ornatus* Boh., 1829*Bruchus vicinus* Gylh., 1833*Bruchus ambiguus* Gylh., 1839*Bruchus sinuatus* Fhs., 1839

5-9 11-19 21 24-27 29-31 34 44
6, 14, 15, 42
10
Not - B. A. C.
Not - B. A. C.

- Callosobruchus chinensis* (L.) *Many teeth*
Curculio chinensis L., 1758 *Not R.A.E.*
Bruchus barbicornis F., 1801 *Not R.A.E.*
Bruchus bistriatus F., 1801 *Not R.A.E.*
Bruchidius thunbergi n.n.
Bruchus chinensis Thnb., 1816 (nec Linnaeus, 1758) *Not R.A.E.*
Bruchus longicornis Thnb.
Bruchus longicornis Thnb., 1816 *Not R.A.E.*
Bruchus litteratus Schönh., 1833 *Not*

Main Differences between *C. maculatus* and *C. analis*.

The original descriptions of *C. maculatus* and *C. analis* are based on the general ground colour and on the pattern formed by the pubescence of the two species.

The ground colour of the cuticle varies considerably in *C. maculatus*. One form is black over most of its body, whereas another extreme form is entirely testaceous and closely resembles *C. analis* in this character. Both forms have been found among wild and cultured specimens. The type specimen of *C. maculatus* is of the form with testaceous antennae, whereas that of *C. quadrimaculatus* is of the darker form.

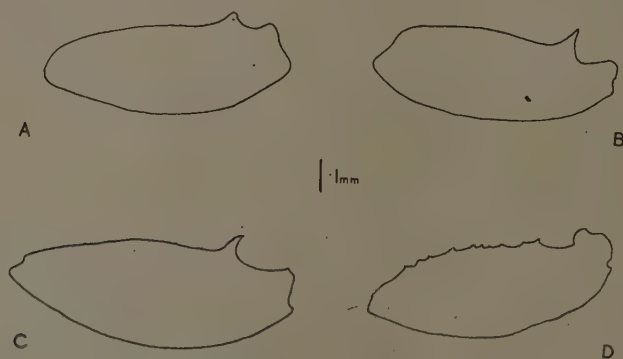


Fig. 1.—Drawings showing position of denticles on hind femora. A, outer tooth of *C. maculatus*; B, inner tooth of *C. maculatus*; C, outer tooth of *C. analis*; D, inner teeth of *C. analis*.

In freshly emerged specimens, the colour patterns of the two species are sufficiently distinct to separate them, the obvious difference being that the white scales are conspicuous in *C. analis* whereas in *C. maculatus* they are relatively inconspicuous (Plate IV). The disposition of the darker areas of elytral cuticle and the white and golden pubescence, however, is almost identical in the two species. It is thus impossible to name old and rubbed specimens on the basis of cuticle colour and pattern, and it is not difficult to understand why these species have been confused. They can, however, be distinguished most reliably by examining the hind femur (fig. 1), which bears a large spine on its inner carina in *C. maculatus* and only a small one, or even none at all, in *C. analis*.

Some other external differences are listed in Table I and mentioned in the description given later, but none of these is as clearly defined as the femoral character. The genitalia of both species were therefore examined in the hope of finding further differences. The male genitalia (figs. 2-4, and Pl. III, figs. 5, 6)

were found to exhibit a very obvious difference in the endophallic armature as well as several other differences of detail. The females were readily separated by their genitalia which showed a difference in the armature of the bursa (fig. 5). Unfortunately, the latter is sometimes missing from dried specimens.

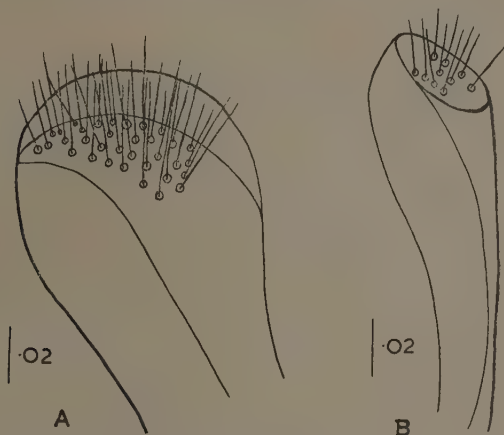


Fig. 2.—Tip of paramere of A, *C. maculatus*; B, *C. analis*.
Scale: mm.

The very large numbers of *C. maculatus* that we have examined show a very wide range of colours, from specimens that are brown overall, with little or no pattern on the elytral cuticle, to specimens with a very definite pattern. This variation is related to sex, the female usually being maculate and the male plain, but there is a wide range of intermediate forms. In *C. maculatus* it is possible to separate the sexes on elytral and pygidial markings, but in *C. analis*

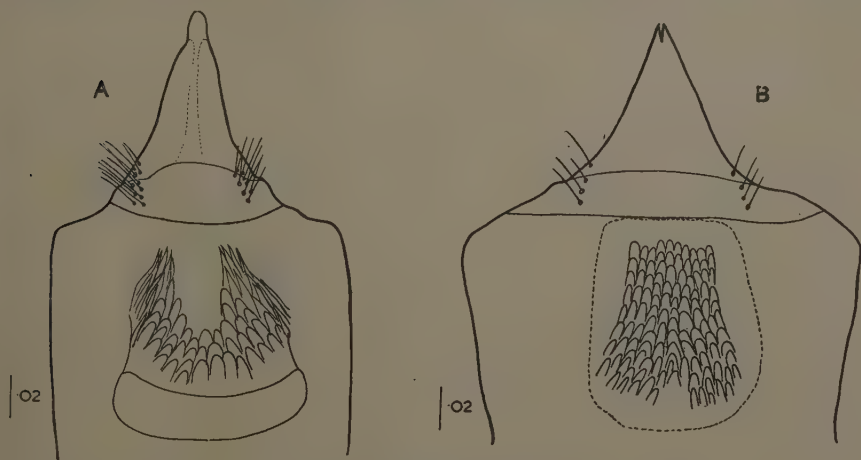


Fig. 3.—Tip of median lobe of A, *C. maculatus*; B, *C. analis*.
Scale: mm.

this is much more difficult. The elytral markings of *C. analis* are more constant and are similar in both sexes. Both sexes also have a median line of white pubescence on the pygidium. It is possible, however, to separate them by the cuticular colour of the pygidium, which bears a wide median testaceous stripe or V-shaped area in the male and a much narrower one in the female.

TABLE I.

Comparison of the important distinguishing features of *Callosobruchus maculatus* (F.) and *Callosobruchus analis* (F.).

Character	<i>Callosobruchus maculatus</i>	<i>Callosobruchus analis</i>
Antennae	Subserrate	Not serrate
Eyes	Deeply emarginate, prominent, bulbous	Less deeply emarginate, flattened, less prominent
Thorax	Median lobes extending well beyond posterior margin	Median lobes extending only slightly beyond posterior margin
Hind femur	Large, acute tooth on inner carina of ventral edge	Tooth small or absent on inner carina of ventral edge
Parameres	Spatulate at tip, large numbers of setae at apex	Slightly spatulate, small numbers of setae at apex
Median lobe of male genitalia	Endophallus lined with large numbers of heavily chitinised denticles Chitinised area below exophallic orifice deeply emarginate apically	Endophallus lined with few denticles Chitinised area below exophallic orifice rectangular

The colour variation of *C. maculatus* is due neither to the food on which the particular specimens were bred, nor to their geographical origin. A wide range of variation may be exhibited by the offspring of a few individuals bred on a single foodstuff under normal conditions. In *C. maculatus* there is a form in which the



Fig. 4.—Chitinised plates in the saccus region of A, *C. maculatus*; B, *C. analis*. Scale: mm.

female bears pale grey pubescence with a very prominent black spot. This has been received from several countries, including Nigeria, where Mr. G. Caswell of Ibadan University has found a very active, strongly maculate form with a white pygidium, which he believes to be migratory and which is apparently sterile in culture (see p. 86). Utida (1954) has also described a "flight" form with low fertility.

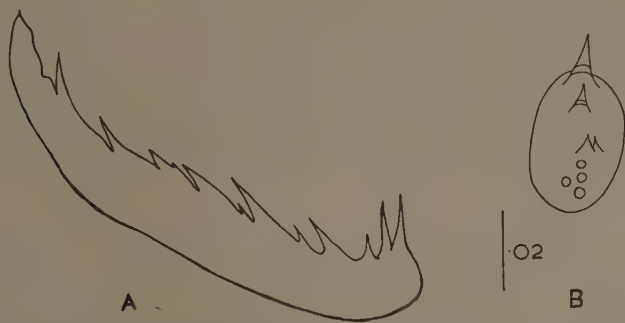


Fig. 5.—Chitinised area on the bursa of A, *C. analis*; B, *C. maculatus*.
Scale: mm.

Descriptions of Species.

Callosobruchus maculatus (F.) (Pl. IV, figs. 1–3). Antennae slightly serrate from the fourth to the apical segment (Pl. III, fig. 1). Segments 3–5, usually basal 4, testaceous, the rest black (specimens with completely testaceous antennae have been seen in the samples examined). Head black, confusedly punctate, sparsely covered with fine, golden pubescence; median carina not prominent, extending from just in front of the eye to just behind. Eyes bulbous, prominent, and deeply emarginate, antennae inserted at mouth of emargination (fig. 6, A). Thorax black, evenly rounded, confusedly punctate, sparsely covered with golden pubescence; basal median lobes prominent, extending well behind the posterior border of the thorax, completely covered with white, scale-like pubescence. Scutellum covered with white, scale-like pubescence. Elytra together slightly longer than broad; striate (Pl. III, fig. 3), striae punctured, punctures deep, each projecting posteriorly into a shallow tail which extends as far as next puncture, so that sides of striae appear slightly irregular; humeral callosities fairly prominent; elytral pattern usually containing three dark spots, a small humeral, a large median and an apical one. In the male the ground colour is black along the lateral margins and apices, elsewhere testaceous, bearing a pubescence consisting chiefly of golden, scale-like hairs with some white hairs, median black spot when present limited to outer six interstices. In the female the ground colour is black along the sutural margins as well as the lateral margins, with a median black bar across the centre connecting the two black margins; the pubescence grades from white to golden and emphasises the maculate areas of the elytra. Pygidium strongly convex at the sides, projecting beyond elytra, oblique; in the male, ground colour wholly black, or testaceous with margins and median line black, and bearing a fawn or grey pubescence; in the female, ground colour testaceous, median line bearing a white pubescence which may extend beyond the median area. Legs testaceous, femora of hind legs bicarinate ventrally, with a large blunt tooth on the outer carina (fig. 1, A) and a sharp tooth of similar size on the inner carina (fig. 1, B) both situated near the apex.

"Active" form of *Callosobruchus maculatus* (F.).—Amongst the many forms produced by this very variable species is one that appears to be less fertile and is possibly migratory (Pl. IV, figs. 4 & 5). The female is characterised by a ground colour that is almost wholly black. This causes the median black spots on the outer margins of the elytra to be very prominent, and the uniform covering of white and golden pubescence to appear grey. The disposition of the white and golden scale-like hairs is similar to that of the usual form but is much more sharply defined. The white hairs bounding the median black spot are disposed as a longitudinal band of white pubescence on the third interstice marking the internal edge, with a very narrow transverse band on the 4th–9th interstices forming the anterior edge and with a wider band across the same interstices forming the posterior edge. Ground colour of pygidium testaceous at apex, pubescence either entirely white, or golden with a narrow median line of white. Hind legs black or very dark testaceous, other legs testaceous. These characteristics are also apparent, though less well defined, in the male of this form.

Callosobruchus analis (F.) (Pl. IV, fig. 6). Antennae not serrate, wholly testaceous* (Pl. III, fig. 2). Head testaceous, confusedly punctate, sparsely covered with fine, golden pubescence, median carina visible, but not prominent, extending from just in front of the eyes to just behind. Eyes flattened and not prominent, emarginate around antennal insertions but less deeply so than in *C. maculatus*, antennae inserted just in front of the eye (fig. 6, B). Thorax

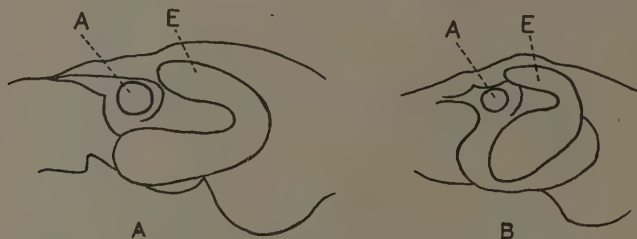


Fig. 6.—Insertion of antennae of A, *C. maculatus*; B, *C. analis*.
A, place of insertion; E, eye.

testaceous, evenly rounded, confusedly punctate, with very sparse, golden pubescence, basal median lobes not prominent and not extending behind posterior border of thorax, covered with a white, scale-like pubescence. Scutellum covered with inconspicuous, fine, golden pubescence. Elytra together slightly longer than broad, striate (Pl. III, fig. 4), punctate, striae shallow and straight, humeral callosities fairly prominent; elytral pattern and arrangement of pubescence basically the same as in the female *C. maculatus*, but the pubescence somewhat finer; ground colour testaceous with dark areas apically; within the distal half of each elytron, the white scale-like pubescence invariably forms a prominent spot on an area of testaceous cuticle which is more or less surrounded by dark cuticle. Pygidium strongly convex at sides, projecting beyond elytra, oblique, ground colour varying from testaceous with two black patches to entirely black with a white pubescent median line; the remainder of the pygidium covered with white—golden pubescence. Legs testaceous, femora of hind leg usually bicarinate ventrally, with a large pointed tooth on outer carina (fig. 1, C), tooth on inner carina very minute or absent. On a slide mount a number of small denticles can be seen along the edge of the inner carina (fig. 1, D) on the basal half of the femur.

* One specimen has been seen with the basal four joints testaceous and the rest black.

Descriptions of the male Genitalia.

These descriptions adopt the terminology of Mukerji & Chatterjee (1951) who have described and figured the genitalia of *C. analis*. In *Callosobruchus* the lateral lobes or parameres are finger-shaped and joined at the base to form a U-shaped structure (Pl. III, figs. 5, 6). The median lobe comprises an exophallic tube some 2 to 3 times as long as broad, surrounding an eversible endophallus which is armed with denticles. The tip of the exophallus is surmounted by a triangular lip or valve (fig. 3) in front of the exophallic orifice. Basally, the exophallus bears a lightly chitinated apodeme with two strengthening prongs. The lateral arches and hypomere are not easily seen in some preparations, being obscured by the endophallic armature. The endophallus is armed internally with denticles (Pl. III, figs. 5, 6) which may form a definite pattern constant for the species; it also bears apically a small but very distinct area of chitinated pointed denticles (fig. 3). Basally there are two oval chitinated plates bearing short stout denticles (fig. 4).

Callosobruchus maculatus (Pl. III, fig. 5).

The parameres are quite straight and narrow, extending to the apex of the exophallic valve or slightly beyond. The apex of each paramere is distinctly broadened into a spatulate apex which bears about 36 setae (fig. 2, A).

The chitinous area at the apex of the endophallus is emarginate and thus approximately U-shaped (fig. 3, A). The spines lining the endophallus are large and heavily chitinated basally, gradually changing to granulate and lightly chitinated distally. Two very distinct lateral, heavily chitinated, granular areas are characteristic of this species (Pl. III, fig. 5). The exophallic valve bears two rows of 4-6 setae on each side (fig. 3, A).

Callosobruchus analis (Pl. III, fig. 6).

The parameres are distinctly curved towards the median line in their apical third and extend beyond the apex of the exophallic valve. The apices are only slightly spatulate and bear about ten setae on the apex (fig. 2, B). The chitinous area at the tip of the endophallus is rectangular, often narrowly or broadly emarginate along the basal border (fig. 3, B). The spines lining the endophallus are lightly chitinated throughout and bear distinct pointed teeth only in the area of the saccus (fig. 4, B). There are no heavily chitinated areas at the sides of the endophallic tube (Pl. III, fig. 6). The exophallic valve bears only one row of 3-4 setae on each side (fig. 3, B).

Comparison of female Genitalia.

One difference has been noted in the female genitalia of the two species. The bursa copulatrix in both species is a pear-shaped bag which bears a pair of thin-walled, cup-shaped structures near the middle. Between these cups there is in each species a distinctive armature. In *C. maculatus*, there are up to four chitinated teeth, apparently inserted on or near to an oval plate (fig. 5, B). In *C. analis*, there is an irregularly toothed ridge, about the same length as the cups and with its base parallel to the long axis of the bursa (fig. 5, A). The bursa often disintegrates in dead material, but is sometimes of use in the separation of the two species.

Summary.

Two of the species in *Callosobruchus*, *C. maculatus* (F.) and *C. analis* (F.), are compared and redescribed, together with a description of the male and female genitalia of both species. The species *Bruchus vicinus* Gylh., *B. ornatus* Boh., *B. ambiguus* Gylh., *B. sinuatus* Fhs. and *B. quadrimaculatus* F. are shown to

be synonymous with *C. maculatus* (F.). *Bruchus jekeli* Allib. and *B. glaber* Allib. are almost certainly synonyms of *C. analis* (F.), but this could not be verified as no type specimens are available.

Acknowledgements.

Our thanks are due to the British Museum (Natural History) and the Commonwealth Institute of Entomology for the willing help given by their staff, especially Mr. R. D. Pope, who examined the type of *C. analis* and gave other valuable assistance.

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FIG. 1. Antenna of *Callosobruchus maculatus*.



FIG. 2. Antenna of *Callosobruchus analis*.



FIG. 3. Elytra striae of *C. maculatus*.



FIG. 4. Elytra striae of *C. analis*.



FIG. 5. Male genitalia of *C. maculatus*.



FIG. 6. Male genitalia of *C. analis*.



FIG. 1. Typical female of *Callosobruchus maculatus*.



FIG. 2. Typical male of *Callosobruchus maculatus*, showing inconspicuous spots.



FIG. 3. Typical male of *C. maculatus*, showing spots in the most conspicuous form.



FIG. 4. Female of "active" form of *C. maculatus*.



FIG. 5. Male of "active" form of *C. maculatus*.



FIG. 6. *Callosobruchus analis*.

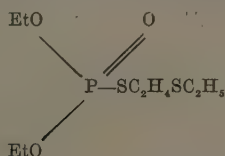
INSECTICIDAL ACTION STUDIES WITH DEMETON-O AND DEMETON-S.

By W. A. L. DAVID

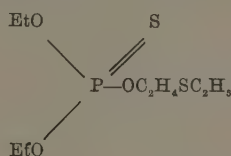
A. R. C. Unit of Insect Physiology, Cambridge.

E.M.N.

The systemic insecticide which has been given the coined name demeton consists of two isomers, demeton-O and demeton-S.



O:PSC
demeton-S
diethyl S-2-ethylthioethyl
phosphorothiolate



S:POC
demeton-O
diethyl 2-ethylthioethyl
phosphorothionate

This mixture of isomers first became available from the Farbenfabriken Bayer Company of Leverkusen under the trade name "Systox". Analyses of a sample showed it to consist of 18 per cent. demeton-O and 30 per cent. demeton-S (Gardner & Heath, 1953), the remainder being emulsifier. The emulsifier is necessary for most practical purposes since the solubility of the isomers in water is low: that of demeton-O (Systox) is 1:15,000 and that of demeton-S (isomeric Systox) 1:500 (Tietz, 1954). The value for demeton-O does not agree with that given by Schrader (1952, p. 78) but has been confirmed in correspondence with the Bayer Company.

Within the plant both isomers are changed into other compounds so that it is largely metabolites derived from them that reach the insects (Metcalf & others, 1954; Heath, Lane & Park, 1955).

The present paper reports some comparative tests of the insecticidal effect on Aphids of plants treated with these two compounds. In addition, demeton-S containing ^{32}P has been used to study the uptake of this material by plants and the distribution of the radioactive decomposition products to which it gives rise. In all solutions the emulsifier, Bayer 8139, described as a polyoxyethylene alkyl aryl phenol, was used.

Methods.

For the tests, broad beans, *Vicia faba*, infested with *Aphis fabae* Scop., were used. These plants were grown in John Innes pasteurised potting compost or in sand, and during the experiments they were kept in the greenhouse previously described (David & Gardiner, 1951) where the temperature usually fluctuated between 17 and 23°C. As bean plants will not grow well, especially in the early stages, at this temperature, and because of the shortage of greenhouse space, the test plants were raised in another house. This was open in hot weather and in cold weather it was heated sufficiently to keep out the frost. Because of these seasonal variations in temperature and also daylight intensity variations, plants grown and used for tests at different times of the year may be expected to behave

somewhat differently in the tests. The dates of the experiments are therefore given.

After the insecticidal solution had been applied, watering was controlled and none drained away from the pots.

The aphid population on the plants was assessed as usual by classifying it as normal, declining, isolated or nil (abbreviated to N, D, I or O, respectively, in the Tables in the text) in the way which has been described on several previous occasions (David & Gardiner, 1951, 1954). As usual, the persistence of the material was also investigated by attempting to reinfest the treated plants (when they were free of Aphids) on the fifth or tenth day after the beginning of the experiment. Control plants were kept in all experiments and on these the Aphids always remained normal. The radioactivities of the demeton-S solutions were assayed on standard Geiger-Müller counting equipment using a liquid-type counter (Veall, 1948). The plant material was prepared for the counter by chopping and boiling with 5 per cent. sodium hydroxide. The boiling was stopped when the volume was reduced to about half and the remaining solution was diluted and allowed to stand before assaying. Demeton-S hydrolyses readily in alkaline solution and all the phosphorus was present as ionic compounds. The radioactivity determined in the plant material has been calculated as demeton-S though, as explained, this material is rapidly broken down in the plant, forming toxic and then non-toxic compounds. For this reason the radioactivity is not a reliable measure of toxicity especially when some days have elapsed between the treatment and the assay. The radioactivity of the solutions used in the experiments varied considerably and is indicated for each experiment.

RESULTS.

Dipping Tests.

The bean plants were trimmed so as to leave only a single pair of well-grown, undamaged leaves. These were infested with Aphids on day 0 and dipped on day 1 as previously described (David & Gardiner, 1951, 1954). The results show that when the plants were infested before dipping, demeton-S was about ten times as effective as demeton-O and was also more persistent (Table I).

TABLE I.

Average results of three dipping tests with solutions of demeton-O and demeton-S containing 0.1 per cent. of emulsifier at 20°C. (7.iii.53, 24.ii.54 and 7.iv.54).

Material	Concentration (% v/v)	Number of days to kill Aphids when plants infested on day		
		0	5	10
Demeton-O . . .	0.10	1	3	7
	0.01	> 10	—	—
	0.001	> 10	—	—
Demeton-S ..	0.10	1	2	3
	0.01	1	5	> 10
	0.001	1	> 10	—
	0.0001	> 15	—	—
Control	0.1% emulsifier	> 15	—	—

Absorption and Systemic Action following Applications made to the Roots.*(a) Plants in solutions.*

Bean plants about 4-6 in. high growing in sand were infested with Aphids on day 0 and the next day or the following, in either case called day 1, when the colonies were established, the plants were unpotted and the roots washed. The roots were then placed in 100 cc. of solutions of the insecticides contained in half-pint milk bottles. The necks of the bottles were plugged with cotton wool.

TABLE II.

Average results of three tests in which the roots of bean plants were placed in solutions of the insecticides (10.iv.54, 16.ii.55 and 5.iv.55).

Material	Concentration (% v/v)	Number of days to kill Aphids when plants infested on day	
		0	5
Demeton-O	0.01	2	3
	0.001	5	4
	0.0001	>10	—
Demeton-S	0.001	2	2
	0.0005	2	4
	0.0001	4	>5
	0.00001	>10	—

After five days the bottles were washed and the solutions renewed. The state of the Aphid population on each plant was noted daily and the results obtained again indicated that demeton-S was about ten times as toxic as demeton-O (Table II).

This experimental procedure was used with demeton-S containing ^{32}P to investigate the uptake of radioactive material from solutions and its distribution in the treated plants.

TABLE III.

The absorption of demeton-S from solutions by the roots of broad beans.

Date	Conc. (% v/v)	Activity of soln. (approx.) ($\mu\text{c./l.}$)	Vol. absorbed (ml.)	Activity gain or loss (%)	Vol. absorbed (ml.)	Activity gain or loss (%)	Vol. absorbed (ml.)	Activity gain or loss (%)
			Day 2		Day 4		Day 5	
27.vii.53	0.001	2	37	0	64	31	—	—
	0.0005	1	31	— 3	55	27	—	—
14.viii.53	0.005	4	20	—13	53	3	86	39
	0.001	< 1	21	— 2	53	11	92	93
			Day 2		Day 3		Day 4	
1.v.54	0.0005	4	43	8	67	40	75	42
	0.00005	< 1	41	5	64	38	69	39
15.v.54	0.0005	2	39	— 3	56	17	88	200
	0.00005	< 1	33	— 5	52	19	62	75

Vol. absorbed = average ml. of solution absorbed by three plants from the 100 ml. supplied to each. Activity gain or loss = per cent. increase or decrease in activity of solution.

The results obtained are shown in Table III. Each value is based on the assay of the solution from three plants except on day 4 and 5 when it sometimes happened that all the solution had been absorbed by some of the plants. It will be noted that on the second day, when only low volumes of solution have been absorbed, there was in general a decrease in activity of the solution indicating a selective absorption of the demeton-S, but that on subsequent days the activity of the solution increased indicating a selective rejection of the demeton-S. A similar selective absorption followed by rejection has been recorded for schradan (David, 1951) and for demeton-O (Tietz, 1954)*.

Some of the bean plants in the foregoing experiment and others similarly treated were used to investigate the distribution of radioactive material in the plant after two and four days at different concentration levels. The effect on the Aphid population was also noted in some experiments.

TABLE IV.

The distribution of radioactive material between the roots, beans and aerial parts of broad bean plants which have absorbed solutions of demeton-S through the roots. Each value is the average for two plants.

Date	Conc. (% v/v)	Activity of soln. (approx.) (μ c./l.)	Sampled on day	Aphid popln. on day sampled	Radioactivity as mg. of demeton-S per kg. of fresh plant tissue in :			
					Root	Bean	Stem	Leaves
27.vii.53	0.001	2	4	—	15.0	2.0	29.0	
	0.0005	1	4	—	8.0	1.0	13.0	
14.viii.53	0.005	4	4	—	58.0	38.0	121.0	
	0.001	< 1	4	—	13.0	8.0	25.0	
1.v.54	0.0005	4	2	I	5.0	0.4	2.8	10.7
			4	O	8.0	1.1	3.3	18.2
	0.00005	< 1	2	N	0.6	0.1	0.3	1.2
			4	D	0.8	0.1	0.3	2.0
15.v.54	0.0005	2	2	I	4.4	0.2	5.8	
			4	O	9.0	0.5	12.9	
	0.00005	< 1	2	N	0.4	—	0.6	
			4	D	0.9	0.3	1.2	

Aphid population: N = normal, D = declining, I = isolated individuals only, O = no Aphids.

From the results given in Table IV it may be seen that radioactive material passed to all parts of the plants and that there was a higher concentration in the aerial parts taken as a whole than in the root. In this respect demeton-S differed from schradan and dimefox (David, 1951, 1952) but resembled demeton-O (Tietz, 1954). Furthermore, leaf samples were more active than stem samples.

* Since this paper was submitted for publication in November 1955, Thomas, Bennett & Lloyd-Jones have described investigations carried out by them (December 1955). For the most part the two investigations cover different aspects of the problem. Where they cover the same ground the conclusions are similar. On one point there is a difference. In the present paper it is accepted that Tietz (1954) was, as he claimed and has subsequently confirmed in a personal communication, working with pure demeton-O isomer. Thomas, Bennett & Lloyd-Jones, however, conclude that he was using the mixed isomers and say on page 587 "Tietz (1954), using the ^{32}P -labelled active ingredient E1059 (shown by Gardner & Heath (1953) to consist of both demeton-O and demeton-S isomers) . . .". In a personal communication they have given their reasons for this interpretation. It seems that there remains some uncertainty which cannot readily be resolved.

It might be thought that this difference was largely accounted for by differences in the dry-matter content of the two organs but this was not so (stem dry matter = 14.0%, leaf 16.0%). It must be concluded therefore that there was a higher concentration of active material in the leaves than in the stems. The Aphids showed a toxic effect when radioactivity equivalent to 1-2 mg./kg. of demeton-S was present in the aerial parts.

(b) *Demeton-O and demeton-S administered by the cut tap root.*

A known dose of insecticide solution was administered to a bean plant by cutting the tap root at a suitable point and placing the cut end in a small tube containing the insecticide. The remaining lateral roots of the plant were allowed

TABLE V.

Bean plants given 1 ml. each of solutions of demeton-S containing ^{32}P by the cut tap roots (12.v.54).

Concentration (% v/v)	Dose given to plant (calc.) (mg./kg.)	Condition of aphid colony when sampled (% remaining)	Concentration of radioactive material in plant calculated as mg. of demeton-S per kg. of fresh plant tissue		
			In stem and leaves	In root	Average whole plant (calc.)
0.01	4.6	<50	5.7	2.7	4.2
	4.3	<50	5.3	3.3	4.3
0.005	2.2	50-75	2.5	1.5	2.0
	2.2	50-75	2.2	1.9	2.1
0.0001	0.5	100	0.5	0.5	0.5
	0.5	100	0.6	0.4	0.5

Activity on 12.v.54 of 0.01% solution = 40 $\mu\text{c.}/\text{l.}$ approx.

to take up water. A 1 ml. dose was taken up in about 2-3 hours when transpiration was rapid (David & Gardiner, 1951). When tested in this way the lowest dose of demeton-O which killed all the Aphids on the treated plant was about 3 mg. per kg. fresh plant tissue while 1 mg./kg. was without effect. With demeton-S, 1 mg./kg. killed all Aphids but 0.5 mg./kg. was without effect in 10 days. Unfortunately the repeatability of the results obtained in six tests was too poor for a more precise comparison of the toxicity of the two compounds to be possible.

TABLE VI.

Comparison of the insecticidal activity of demeton-O and demeton-S watered on to soil in which bean plants were growing (16.ii.55 and 18.iii.55).

Concentration (% v/v)	Number of days to kill Aphids when plants infested on day			Number of days to kill Aphids when plants infested on day		
	Demeton-O			Demeton-S		
0.10	4	2	2	1	2	2
0.01	7	5	6	4	4	5
0.001	>10	—	—	>10	—	—

TABLE VII.

The uptake of demeton-S containing ^{32}P and its distribution in broad-bean plants growing in sand or soil watered with the radioactive solutions.

	Day 2				Day 6					
Conc. (% v/v)	Leaves (mg./kg.)	Stem (mg./kg.)	Aphids on day* 2	Root (mg./kg.)	Leaves (mg./kg.)	Stem (mg./kg.)	Aphids on day 2 5	Root (mg./kg.)		
(a)	Sand				Sand					
0.05	118 158	41 48	I O	40 53	437 457	40 43	O O	55 50		
0.01	28 24	10 9	I I	10 9	68 83	6 9	I I	O O	13 16	
0.005	1.6 1.3	— —	D D	1.2 0.9	33 6.2	— —	D D	I I	1.3 2.1	
	Soil				Soil					
0.05	36 42	13 17	I I	10 14	76 50	12 9	I O	O O	14 11	
0.01	2.7 3.1	1.3 1.0	D I	1.3 1.5	6.6 10.9	1.2 1.6	D D	O O	2.0 2.8	
0.005	0.2 0.3	— —	N N	0.3 0.3	0.5 0.6	— —	N N	N N	0.2 0.3	
	Leaves & stem (mg./kg.)				Leaves & stem (mg./kg.)				Aphids on day 2 5	Root (mg./kg.)
(b)	Sand				Sand					
0.01	20.3 26.2		I I	12.9 19.7	57.0 55.2		I I	O O	19.4 18.3	
0.005	14.3 19.3		I I	10.5 11.5	20.7 30.2		O I	O O	7.4 6.7	
0.001	2.3 2.2		D D	2.8 2.5	4.6 6.4		D D	I O	1.6 2.3	
0.0005	0.9 0.9		N N	1.3 1.0	2.2 1.6		N N	I I	0.8 0.9	
	Soil				Soil					
0.01	2.6 3.9		D I	— —	10.4 13.1		I D	O O	— —	
0.005	2.5 3.7		D I	— —	5.2 7.0		D D	D I	— —	
0.001	0.2 0.4		N N	— —	1.4 0.9		N N	D N	— —	
0.0005	0.02 0.08		N N	— —	0.26 0.15		N N	N N	— —	

The quantity of radioactive material found in the plant is expressed as mg. of demeton-S per kg. of fresh plant tissue (a) Activity on 23.vi.54 of 0.05% solution = 30 $\mu\text{c./l.}$ (b) Activity on 11.v.54 of 0.01% solution = 48 $\mu\text{c./l.}$

* For explanation of symbols, see Table IV.

The same method was used to treat six broad-bean plants with radioactive demeton-S. The solutions were absorbed in two hours and the experiment was allowed to run until the Aphids began to fall off the plants treated with the two highest doses. At this time, 4-4½ hours after treatment, the plants were removed for assay.

From Table V it can be seen that Aphids were affected in 4 hours by doses of 2.2 mg./kg. applied and when the concentration of radioactive material in the aerial parts calculated as demeton-S was about the same. This value may be compared with the observation that a dose of 1.0 mg./kg. of demeton (mixed isomers) killed all Aphids on bean plants in ten days (David & Gardiner, 1954).

(c) *Plants growing in sand or soil.*

The two demeton isomers were compared by watering freshly prepared solutions on to the soil in pots in which broad beans were growing and observing the effect on aphid colonies on the foliage. In these experiments 20 cc. of solution were watered on to 400 gm. of moist soil in 3½ in. diameter pots. The plants in all pots were 4-6 in. high when treated. The experiments were carried out in duplicate and repeated with the average results shown in Table VI. It again appeared that, just after treatment, plants given demeton-S were several times as toxic to the Aphids as those treated with demeton-O, though this difference tended to disappear later.

Demeton-S containing ^{32}P was used to study the absorption of the material by the roots of broad bean plants from treated sand and soil. The pots again contained 400 gm. of moist soil while the moist sand weighed about 570 gm. Like schradan and dimefox (David, 1951, 1952), demeton-S is more readily absorbed from sand than from soil (Table VII). As in the case of plants in solution,

TABLE VIII.

The uptake of demeton-S containing ^{32}P by broad-bean plants grown in sand and repotted into sand or soil.

Concentration (% v/v)	Day 2 Leaves & stem (mg./kg.)	Day 6 Leaves & stem (mg./kg.)	Day 5 Aphid population*
0.01	Sand	Sand	
	15.4	32.7	O
	15.4	42.2	O
0.005	1.1	22.3	I
	2.0	14.8	I
0.001	3.3	1.6	I
	0.2	3.8	I
0.01	Soil	Soil	
	1.1	1.2	N
	1.6	6.1	D
0.005	0.2	4.0	D
	0.1	3.3	D
0.001	0.3	1.3	N
	0.1	0.6	N

The quantity of radioactive material found in the plant is expressed as mg. of demeton-S per kg. of fresh plant tissue. The aphid population was normal on day 2 in all cases. Activity on 20.v.54 of 0.01% solution = 48 $\mu\text{c.}/\text{l.}$

* For explanation of symbols, see Table IV.

combined leaf and stem samples contained more radioactive material per unit weight than root samples and leaf samples were much more active on the same basis than stem samples.

The foregoing experiments were carried out with plants which had been grown throughout in sand or soil. As the root development was not equal in the two cases, the experiments were repeated using plants which had all been grown in sand until they were about 4-6 in. high. At this stage 12 uniform plants were repotted into compost and 12 into fresh sand both in 5-in. diameter pots. Two days later both batches were treated with the solutions of radioactive demeton-S. Two days after treatment half of the plants were cut for assay and a further four days later the remaining plants were cut. The results obtained (Table VIII) confirm that when radioactive demeton-S is watered on to sand or soil in which beans are growing the plants in sand become more radioactive and more toxic to Aphids than the corresponding plants in soil.

Absorption and Translocation within the Plant following Application to the Leaves.

It has been shown previously that when demeton was applied to the older leaves of bean plants, toxic material was usually translocated to the younger

TABLE IX.

Systemic action with demeton-O and demeton-S after treating the older leaves of broad-bean plants once by brushing on the solutions, leaving untreated the Aphid-infested, unexpanded leaves of the growing points.

Material	Conc. (% v/v)	Date	No. of leaves treated		Aphids on untreated leaves			
					First day on which :			
					Declining		None	
			(a)	(b)	(a)	(b)	(a)	(b)
Demeton-O	0.50	14.iv.53	4	4	1	1	2	2
		13.i.54	3	4	>10	>10	—	—
		23.ii.54	5	5	>10	4	—	>10
	0.25	14.iv.53	4	4	1	1	2	2
		25.iv.53	5	4	1	1	4	>10
		8.v.53	4	4	>5	>5	—	—
		13.i.54	3	4	>10	>10	—	—
		8.vii.55	5	5	>5	>5	—	—
		13.vii.55	5	5	1	1	3	3
	0.5	13.i.54	3	4	3	>10	>10	—
		23.ii.54	5	5	>10	>10	—	—
		11.v.54	3	3	1	1	1	3
		4.vi.54	4	4	1	1	3	2
		7.vi.54	4	4	1	2	2	3
	0.25	14.iv.53	4	4	1	1	2	2
		25.iv.53	5	4	1	1	>10	3
		8.v.53	4	4	>5	2	—	>5
		13.i.54	3	4	4	>10	>10	—
		3.vi.54	4	4	1	1	3	3
		8.vii.55	5	5	1	1	3	5
		13.vii.55	5	5	>5	4	—	>5

(a) and (b) are duplicates. Under the column headed "none" — indicates that this condition was not reached as the test was stopped on the day shown under "declining" when the population was still normal.

untreated leaves in sufficient quantities to kill Aphids, although occasionally Aphids remained unaffected (David & Gardiner, 1954).

A comparison of the separated isomers.

These experiments were repeated with the separated demeton-O and demeton-S and the results obtained are shown in Table IX. Like demeton, when applied to the older leaves, the separated isomers killed Aphids on untreated younger leaves but the results were again variable and accurate comparisons between the isomers could not be made. Both compounds were toxic at 0.25 per cent., but demeton-S gave the most consistent results.

Translocation experiments with demeton-S containing ^{32}P .

Because of the inconsistent results obtained in the foregoing experiments, the translocation of material from the treated leaves of broad beans to untreated leaves, both above and below them, was investigated in a series of experiments using demeton-S containing ^{32}P . From the results given in Table X it is evident that variable amounts were translocated, but more radioactive material was always moved upwards to younger leaves than downwards to older ones. Of the total material recovered, more than 90 per cent. was still associated with the treated

TABLE X.

Translocation of radioactive material after applying 0.1 per cent. demeton-S and 0.1 per cent. schradan containing ^{32}P to leaves on the middle part of the stem of broad-bean plants.

Sampled on day	Solution applied		Total recovered.	Distribution of recovered material & effect on Aphids				
	Dose	Activity		Treated leaves	Leaves above			Leaves below
	(mg.)	($\mu\text{c./l.}$)	(%)	(%)	(%)	(mg./kg.)	Aphids*	(%)
(a) 4 (23.vii.53)	1	18	34.9 23.7 39.4	97.0 93.7 93.3	2.9 6.1 6.6	2.0 2.6 4.7	N O O	0.1 0.1 0.1
4 (5.viii.53)	1	9	52.5 60.3 54.0 35.6	95.0 95.7 93.5 92.5	4.6 3.8 5.7 7.3	4.2 4.1 6.8 4.4	— — — —	0.4 0.4 0.8 0.3
1 (5.v.54)	5	3480	72.7	96.0	3.2	18.7	—	0.9
1 (11.v.54)	2.5	2380	53.5	97.3	1.8	13.6	I	0.9
1 (4.vi.54)	2.5	1740	62.2 54.6	97.8 97.4	2.2 2.6	8.8 11.2	D I	— —
(b) 6 (6.vii.50)	— —	<10 <10	— —	97.0 93.5	2.5 6.3	4.3 7.1	— —	0.5 0.3

(a) Leaves treated with demeton-S, (b) leaves treated with schradan.

* For explanation of symbols, see Table IV.

leaves. The results obtained with schradan (Table X) were essentially similar (*cf.* Thomas & Bennett, 1954).

The effect of light on translocation.

Heath & Llewellyn (1953) found that both visible and infra-red radiation increased the rate of absorption of schradan and Thomas & Bennett (1954) found that light influenced absorption and translocation. It is shown below that light also influences the translocation of demeton-S.

When bean plants were preconditioned by four days in dim light and the leaves on the middle parts of the stem were treated with radioactive demeton-S and afterwards the plants were kept at the same low intensity, the quantity of ^{32}P translocated in 24 hours was less than in plants kept at normal greenhouse illumination (Table XI).

TABLE XI.

The effect of light on the translocation of radioactive material after applying demeton-S to the leaves on the middle parts of the stem of broad-bean plants.

Light condition		Dose applied		% of applied dose recovered	Distribution of recovered material					
Before treatment	After treatment	Vol. (ml.)	Conc. (%)		Treated leaves		Leaves above		Leaves below	
					(mg./kg.)	(%)	(mg./kg.)	(%)	(mg./kg.)	(%)
5.v.54										
Light	Light	1	0.5	36	352	96	19	3	11	1
Shade	Shade	1	0.5	62	688	99	2	0.1	1	<0.1
11.v.54										
Light	Light	0.5	0.5	26 52	92 218	89 97	21 14	6 2	19 8	5 1
Shade	Shade	0.5	0.5	60 76	265 363	99 99	0.5 1.0	<0.1 0.1	0.3 0.1	<0.1 <0.1

The plants were sampled 24 hours after treatment. The recovered material is calculated as mg. of demeton-S per kg. of fresh plant tissue. 5-6.v.54. Temperature averages 20.8 and 22.8°C., respectively. 11-12.v.54. Temperature averages 25.8 and 21.3°C., respectively.

The experiment just described could be criticised on the grounds that the treated leaves of the plants in light dried much more quickly than those of the plants in shade, and according to Tietz (1954) a difference in the rate of drying influences the relative amounts of ^{32}P subsequently translocated. It is difficult to arrange for equal rates of drying in the two groups without introducing some other variable. If, however, it is the light intensity in the preconditioning period and not in the 24 hours after treatment that determines the amount translocated it should be possible to show a difference if the plants from both light and shade are held in shade after the treatment, when evaporation should be equal.

From the results obtained (Table XII) it can be seen that more radioactive material was translocated in plants kept in normal light before treatment than those kept in dim light, although after treatment, when the translocation was actually taking place, both groups were in dim light (less than 3 foot-candles). The same conclusion has been reached by Thomas & Bennett (1954) working with schradan.

In a further experiment, the influence of different periods of preconditioning was compared. The plants were kept in normal daylight or placed in dim light 1, 2 or 4 days before the demeton-S was applied. After treatment they were kept in the light for 24 hours and then sampled. The results, in Table XIII, show that translocation is reduced by the full amount after one day in dim light.

TABLE XII.

The effect of preconditioning plants in light and shade for 3 days on the translocation of radioactive material after applying 0.5 cc. of 0.5 per cent. demeton-S to the three lower leaves on the stem of broad-bean plants.

Light condition		% of applied dose recovered	Distribution of recovered material			
Before treat- ment	After treat- ment		Treated leaves		Leaves above	
			(mg./kg.)	(%)	(mg./kg.)	(%)
Light	Shade	82	386	99	9	1
		84	356	96	21	4
		83	542	99	9	1
Shade	Shade	72	435	99.9	0.4	<0.1
		54	580	99.9	0.5	<0.1
		84	480	99.8	2.8	0.2

The plants were sampled 24 hours after treatment. The recovered material is calculated as mg. of demeton-S per kg. of fresh plant tissue (10.vi.54).

The Fumigant Action of the Insecticides either directly or from Treated Plants.

It has been shown that demeton solutions give off vapours which are toxic to insects (David & Gardiner, 1954). By enclosing aphid-infested leaves with solutions of demeton-O and demeton-S using the same technique it has now been found

TABLE XIII.

The effect of different periods of preconditioning in dim light on the translocation of radioactive material after applying 0.5 cc. of 0.5 per cent. demeton-S to the four lower leaves on the stem of broad-bean plants.

Days in dark before treatment	% of applied dose recovered	Distribution of recovered material			
		Treated leaves		Leaves above	
		(mg./kg.)	(%)	(mg./kg.)	(%)
0	63	246	97.8	8.8	2.2
	55	202	97.5	11.2	2.5
1	72	304	99.9	1.2	0.1
	72	239	99.8	1.4	0.2
2	62	308	99.8	1.4	0.2
	52	177	99.8	0.9	0.2
4	61	328	99.9	1.0	0.1
	64	262	99.9	1.1	0.1

The recovered material is calculated as mg. of demeton-S per kg. of fresh plant tissue. Activity of solution 8,700 μ c./l. (4.vi.54).

that solutions of both isomers also produce toxic vapours. When compared at 20°C., solutions of demeton-O were slightly the more rapid in action against *Aphis fabae*.

However, the more interesting question is whether plants treated systemically with demeton by the roots give off detectable quantities of toxic material from the leaves.

In the first experiments, solutions of demeton (mixed isomers) were used, but later the separate isomers became available. The method used has already been described and illustrated (David & Gardiner, 1951). Briefly, it consisted of enclosing the foliage of two bean plants (whose stems were about 3 in. apart) in a 10-in. diameter glass cylinder. The roots of one plant were in demeton solution and those of the other in water. The top of the narrow-necked jar containing the solution and the holes in the board which closed the bottom of the cylinder and through which the stems of the plants passed were carefully plugged. The top of the solution jar was at least one in. from the bottom of the board. In this way no vapour could pass from the jar to the cylinder.

TABLE XIV.

The evolution of toxic vapour from the foliage of bean plants treated with demeton, demeton-O or demeton-S via the roots.

Date	Material	Concentration (%)	Aphids on untreated plant	
			First day on which: Declining None	
7.viii.52	Demeton	0.05	2	3
12.viii.52	Demeton	0.05	3	> 5
2.ix.52	Demeton	0.05	—	3
11.ix.52	Demeton	0.05	1	3
2.xii.52	Demeton	0.05	—	1
1952	Water controls	—	> 5	—
14.i.53	Demeton-S	0.05	—	3
	Demeton-O	0.05	3	4
16.x.53	Demeton	0.05	—	3
27.x.53	Demeton	0.05	—	3
1953	Water controls	—	> 5	—
2.iii.54	Demeton-S	0.01	1	2
	Demeton-O	0.01	1	2
1954	Water control	—	> 5	—

The — indicates that the condition referred to was passed over before the first observation or not reached before the last observation. The foliage of the treated plant was enclosed in a glass chamber with the untreated plant. The results given relate to the effect on Aphids on the untreated plant only. In some tests the Aphids were not examined until the third day to allow any toxic material to accumulate and act on the insects.

Both plants were infested with *Aphis fabae*. In experiments with dimefox it has been shown that sufficient toxic vapour is given off by the plants in the solution to kill Aphids feeding on the plant in water (David & Gardiner, 1951). All the experiments which were carried out with demeton and the separated isomers are reported in Table XIV. As the Aphids on the plants in the insecticide solutions were always killed very rapidly the results are not reported. In all experiments carried out at different times of the year sufficient toxic vapour was given off by the treated plants to kill Aphids on the untreated plants. It will be noted that the roots of the treated plants were in solutions; when they were in soil treated

with demeton the Aphids on the untreated plants were not killed—presumably because the demeton was being taken up and given off more slowly.

Several experiments were also run in which attempts were made to collect the toxic material transpired by bean plants with their roots in 0.05 per cent. solutions of demeton. The apparatus used has been described previously (David & Gardiner, 1951). The air stream, which had passed over the plants, was passed through water or through a condensing tube at -70°C ., or through both in succession. The solutions so obtained were tested for toxicity against first-instar larvae of *Aedes aegypti* (L.).

In the first experiment, the solution obtained by bubbling the air stream from the plant chamber through 2 ml. of water for 24 hours was not toxic to the larvae and in the second experiment the collected condensate was not toxic. In the third experiment the air stream was therefore passed over an aphid-infested bean tip. (The cut stem was supplied with water to keep it from wilting.) Altogether in seven out of nine of the remaining experiments the Aphids were killed at the end of a 24-hour exposure. In no case was a solution toxic to mosquito larvae obtained by bubbling the air stream through water, but in four experiments the condensate was toxic to larvae. In one experiment the air stream was first bubbled through water and then passed through the condenser. Only the condensate was toxic. This is interesting since in an experiment with radioactive demeton-S the solution was much more radioactive than the condensate, though neither was toxic to mosquito larvae (Table XV). It is evident that further tests are required to clarify these points.

TABLE XV.

The evolution of toxic vapour from the foliage of bean plants treated with 0.05 per cent. solutions of demeton or demeton-S via the roots.

Material and date	Effect on Aphids in air line	Properties of :			
		Solution	Condensate		
		Radio-activity (cts./min.)	Toxicity to larvae of <i>A. aegypti</i>	Radio-activity (cts./min.)	Toxicity to larvae of <i>A. aegypti</i>
Demeton 6.x.53	—	—	Non-toxic	—	—
Demeton 7.x.53	—	—	—	—	Non-toxic
Demeton 4.iii.54	All dead in 24 hr.	—	—	—	—
Demeton 5.iii.54	All dead in 24 hr.	—	Non-toxic	—	—
Demeton 9.iii.54	All dead in 24 hr. ¹	—	Non-toxic	—	—
Demeton 16.iii.54	Moribund in 7 hr., dead in 24 hr.	—	—	—	Very moribund 5 hr.
Demeton 17.iii.54	All dead in 24 hr.	—	—	—	Very moribund 5 hr.
Demeton 23.iii.54	—	—	—	—	Moribund 5 hr., dead 24 hr.
Demeton 24.iii.54	All dead in 24 hr.	—	Non-toxic	—	Moribund 5 hr., dead 24 hr.
Radioactive demeton-S 12.v.54	—	15.2	Non-toxic	3.0	—
Radioactive demeton-S 1.vi.54	Moribund in 7 hr., dead in 24 hr.	44.6	Non-toxic	1.3	Non-toxic

(1) Ten adults of *Aedes aegypti* enclosed in this line were also all killed.

Discussion.

When leaves or stems of beans or other plants are dipped into solutions of the demeton isomers the insecticides may act as fumigants, as contact poisons, or

systemically as stomach poisons following absorption into the plants. In the latter case the compounds will have been metabolised into something different before they reach the insects. When tested in this way, demeton-S was about ten times as effective as demeton-O, but as explained this was not a simple contact toxicity test. In comparison, when working with adult Greenhouse Thrips, *Heliothrips haemorrhoidalis* (Beh.) and the Citrus Red Mite, *Metatetranychus citri* (McG.), which were placed on dipped mature Valencia oranges, Metcalf & others (1954) found that demeton-S was about five times as toxic as demeton-O to the adults of *M. citri* and three times as toxic to the adults of *H. haemorrhoidalis*.

It also appears that demeton-S is about ten times as toxic as demeton-O to *Aphis fabae* when the compounds are taken up by the intact roots of plants. This too should be regarded as an effective and not an absolute difference in toxicity. In this connection it is known that demeton-S penetrates into the leaves and stems of plants more quickly than demeton-O (Metcalf & others, 1954) and it is reasonable to suppose that the same difference occurs with the roots. Unfortunately radioactive demeton-S was not available in these tests so that no directly comparative tests were made. However, there is evidence from Tietz (1954) that the foliage of beans taking up 0.05 per cent. demeton-O by the roots from solutions contained the equivalent of about 150 mg./kg. after 24 hours. For comparison with the present tests the above value must be divided by ten to determine the quantity which would have been absorbed from an 0.005 per cent. solution—the highest tested. This procedure was justified in the case of demeton-S as can be seen from Table IV. It appears therefore that after 24 hours in an 0.005 per cent. solution, Tietz's bean plants would have contained 15 mg. of demeton-O per kg. of fresh shoot tissue whereas in the present tests with demeton-S the plants contained 121 mg./kg. (Table IV). This difference is unlikely to be accounted for by different rates of absorption in the two sets of plants since Tietz's tests, like those described here, were carried out in well ventilated, shaded greenhouses. There is little doubt therefore that demeton-S is absorbed more rapidly through the roots of broad beans than demeton-O.

When radioactive demeton-O was taken up by the beans from solution, from sand or from soil it was found that when samples were taken on the second and fourth days there was always more radioactive material in the leaves than in the roots. For demeton-O taken up from sand, Tietz (1954) found that at first there was a higher concentration in the roots than in the leaves but, by the 6th day and after, the position was reversed and on the average there was more in the leaves. In the case of beans in soil the concentration of demeton-O in the leaves did not exceed that in the roots until about the 12th day. Tietz explains the high concentration in the leaves by saying that demeton (and presumably its metabolites) travels upwards from the roots in the xylem which terminates in the leaves and so gets concentrated there as the water is transpired. This does not, however, explain the difference between the demeton isomers and schradan with which the concentration in the roots was always higher than in the leaves (David, 1951); it seems therefore that other factors must be involved.

By the cut tap-root technique, the lowest dosages which killed all Aphids on the plants were 3 mg. demeton-O per kg. of fresh plant tissue and 1 mg. per kg. of demeton-S. This ratio is not in agreement with that found by dipping or by root absorption. The explanation may well be the different modes of entry. In the cut tap-root technique the more slowly penetrating demeton-O should not have been at the same disadvantage as in the other tests. The dosages just quoted were obtained following rapid penetration but according to Tietz (1954) after 7–21 days in various plants a concentration of demeton-O equivalent to 8 mg./kg. is no longer toxic to Aphids. By this time, however, the demeton-O will have been converted more completely into toxic and non-toxic metabolites. The toxic doses for Aphids given above show that the demeton isomers are much more toxic to *Aphis fabae*

than to *H. haemorrhoidalis* or *M. citri*. For these pests Metcalf & others (1954) found that the LD100 of the demeton-O isomer metabolite was about 300 mg./kg. and that of the demeton-S isomer metabolite 40 mg./kg.

Regarding leaf applications, the results given in Tables X and XI show that when demeton-S containing ^{32}P was applied to the older leaves of a bean plant, radioactive material was translocated to the rest of the plant, but especially to the younger leaves. The same conclusion was reached by Tietz (1954) regarding demeton-O. In both cases, too, it has been found that the materials translocated may not reach insecticidal concentrations in the untreated parts of the plants, unless the greater part of the plant was treated.

When applied to the leaves the systemic effect of demeton-S was slightly superior to that of demeton-O. Without taking into account differences in toxicity this superiority could be explained by the observation of Metcalf & others (1954) that demeton-S penetrated leaves more rapidly than demeton-O and that the metabolites of the former were the more rapidly translocated.

By dipping bean leaves in 0.05 per cent. demeton-O containing ^{32}P , Tietz found that, after three days, 15.6 per cent. of the recovered material had been translocated. In *Phaseolus vulgaris* and *Tropaeolum majus* after 24 hours only about 1 per cent. had been translocated. In the present tests the corresponding percentage of demeton-S translocated averaged about 2.5 per cent. after one day and 5.2 per cent. after four days (Table X). In contrast, Tietz's value of 15.6 per cent. of the total recovered material translocated for demeton-O seems surprisingly high, especially in view of the relative rates of penetration of the two isomers, and the mobility of their metabolites. It should be noted, however, that the demeton-S was only applied to the upper surface of the leaves, whereas the demeton-O was applied to both surfaces by dipping, and penetration is rather more rapid from under surfaces of leaves (Tietz, 1954).

The demeton isomers have almost equal vapour pressures of about 1.4×10^{-3} mm. Hg./20°C. (extrapolated from Schrader, 1952) and there is general agreement that the mixture—demeton—can act as a direct fumigant under suitable circumstances (David & Gardiner, 1954; Lusi, 1952; Unterstenhöfer, 1952). It has not always been found that plants which have absorbed demeton transpire toxic material from untreated leaves. Lusi (1952) placed the cut stem of an ivy shoot in 0.1 per cent. Systox emulsion and enclosed the foliage in a cylinder; specimens of *Drosophila melanogaster* Mg. were not affected after 24 hours in the cylinder with the shoot. Metcalf & others (1954) working with lemon seedlings and Black Valencia beans and following essentially the procedure previously described (David, 1952) failed to find any evidence of transpired radioactive material after treating the base of the stem, although at the end of the experiments the foliage of the test plants contained from 50–300 mg./kg. of the demeton-O or demeton-S metabolites. Tietz (1954), on the other hand, found that *Coleus* plants with their roots in solutions transpired material which was toxic to flies and gave a condensate toxic to mosquito larvae. The present investigation supports the results obtained by Tietz. There is no doubt that, with broad beans, toxic material is given off by plants absorbing the isomers from solutions since the atmosphere surrounding them becomes toxic to Aphids (Table XIV and col. 2, Table XV). Furthermore, material toxic to mosquito larvae can usually be condensed out of the air stream drawn over the plants (Table XV).

Summary.

The two isomers demeton-O and demeton-S which occur as a mixture called demeton in the commercial insecticide Systox act on *Aphis fabae* Scop. as contact and systemic insecticides and as fumigants.

When applied as a contact insecticide or systemically via the roots from solution or from soil, demeton-S was about ten times as toxic to *Aphis fabae* on

broad beans as demeton-O. Using demeton-S containing ^{32}P it was shown that, when applied to the roots, radioactive material passed to all parts of the plants and that the concentration in the aerial parts was higher than in the roots. Leaf samples were more active than stem samples. By radioassay and by the cut tap-root technique it appeared that the lethal dose of demeton-S was equivalent to about 1.0 mg./kg. of fresh plant tissue. The lethal dose of demeton-O by the cut tap-root technique was 3.0 mg./kg. From solutions, the plants first absorbed demeton-S preferentially, then water preferentially. Demeton-S was more rapidly absorbed from sand than from soil.

Both isomers were translocated from older to younger leaves of broad beans usually in sufficient quantities to kill Aphids but the results were more consistent with demeton-S. The quantity translocated downwards was small. A low light intensity before and after treatment reduced the quantity of demeton-S translocated. There was also a reduction in the quantity translocated when the plants were only shaded before treatment. One day of shading was sufficient to cause the maximum reduction.

Solutions of the two isomers gave off toxic vapours and plants treated via the roots gave off toxic vapours from the foliage.

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PENETRATION THROUGH THE EGG-SHELL OF *PIERIS* *BRASSICAE* (L.).

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The Large White Butterfly, *Pieris brassicae* (L.), is a destructive insect whose life-cycle is well known; it is readily available in laboratory culture. The work described here is intended to be an investigation into such parts of the structure, chemical composition, and modifications of the egg-envelopes during development, as may indicate how toxic materials and simple chemical substances pass from the outside to the living contents. The toxicological work which is described has not been carried out to obtain useful ovicidal materials, but it is hoped that this study may provide a "model" for Lepidopteran eggs in the same way that previous work (Beament, 1946a, 1947, 1951) has outlined the broad principles governing the mechanism of penetration through the eggs of some other Orders.

Eggs were obtained by confining pairs of adults in gauze cages with small potted cabbage plants under a high-intensity tungsten lamp (David & Gardiner, 1952). Batches of up to 90 eggs are laid together by one female, usually on the lower leaf surface, cemented to the leaf at the posterior end of the egg. The members of a batch of eggs all hatch within a remarkably short period of time. In the absence of food, newly hatched larvae eat unhatched eggs and even sluggish larvae. This must have selected strongly for uniformity of development and one must therefore presume that the eggs are fertilised immediately before oviposition. This assumption receives support from the uniformity of the developmental period at a given temperature; regardless of opportunity for oviposition presented to females, no ovoviviparous development has been observed. In work of this type it is extremely important to assess the time of fertilisation, so that embryos may be aged accurately from the time of laying. Also, when fertilisation occurs immediately before laying, one must assume that the micropyle must be free to transmit sperm at this time and also, probably, for at least a short while after oviposition. This would be of considerable importance in showing changes in the resistance of the egg to the penetration of poisons with age. These facts are considered later.

Freshly laid eggs are pale yellow in colour, darkening with embryonic development, and later the embryo can be seen through the shell, which is semi-transparent; the colour and the changes in it are apparently entirely due to the embryo. Each egg has some eleven longitudinal heavy veins, eight of which converge on one central micropyle at the upper (anterior) end (fig. 1, B) but stopping short of the basal (posterior) portion, which is flattened against the leaf (fig. 1, A). Numerous smaller cross veins interconnect the main veins transversely.

The direct removal of eggs by mechanical means only, leads to considerable damage and mortality; it was found that the cement (and leaf wax) could be rapidly softened by applying a small quantity of acetone, thus allowing removal of eggs with a camel-hair brush. This treatment did not lead to any mortality, or observable difference in resistance or rate of development, as compared with eggs naturally attached to pieces of leaf. After removal, batches were kept at 25°C. and 100 per cent. relative humidity until needed, and also after treatment in ovicidal experiments. Under these circumstances, embryonic development is completed in about 100-120 hours.

Morphology of the Egg, and physical and chemical Properties of its Chorion.

Construction of the shell membranes.

The chorion of the egg consists of two fundamental layers, an outer proteinaceous coating, thickened to produce the veins and sculpturing, and an inner layer of tanned protein (fig. 1, D) and sculpturing, and an inner layer, which in eggs of *Pieris* is of a viscous oily material; it was not possible to discover whether this is chorionic, or a product of the oöcyte. Before fertilisation, the lipid layer therefore covers the vitelline membrane (fig. 1, C), which is later transformed into epembryonic material, but is reabsorbed before ecdysis. Over the entire surface of the egg, the female spreads a cement (fig. 1, D), secreted by cement glands. This appears thicker along the sides of veins and other concavities of the shell; it is also the material which fixes the shell to the leaf surface. The chemical and physical properties of these layers will be considered in some detail.

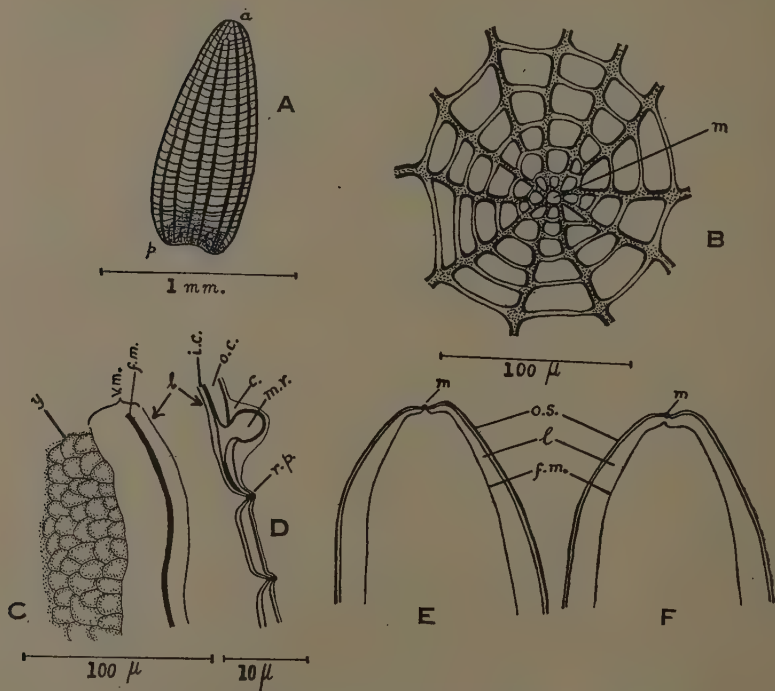


Fig. 1.—A. Whole egg, showing anterior micropylar region (a) and flattened base (p). B. Anterior view of micropylar area and micropylar opening (m). C. Section through inner shell and yolk (y) at about two hours. The fertilisation membrane (f.m.) is developing in the vitelline membrane (v.m.). D. Section showing layers of the outer shell. Cement (c), inner chorion layer (i.c.), main rib (m.r.), outer chorion layer (o.c.), the position of the lipid layer (l) and of a respiratory pore (r.p.) are shown. E. Diagrammatic representation of the fertilisation membrane (f.m.) applied to the micropylar opening (m) in a newly laid egg. F. The same at six hours, showing the retraction of the yolk, allowing the lipid (l) to flow in and isolate the outer shell (o.s.) and micropyle from the fertilisation membrane.

The cement.

The thin layer of cement over the shell of the egg is not present in sufficient quantity to facilitate chemical and physical tests. Much of the work has therefore been carried out on the contents of the cement glands of the female. It was demonstrated that on exposing this material to air, it formed a substance behaving in every way as the layer on the shell, and it seems from this, and evidence given below, that no important chemical addition is made at the time of oviposition. In the gland, the cement is a viscous fluid, consisting of a pale yellow matrix containing large numbers of irregular microscopic granules of similar colour. On exposure it rapidly loses water, becoming more rubbery, and somewhat darker. When completely desiccated, it loses all stickiness and becomes rather brown, but after such treatment it will reabsorb water and recover its initial properties. Although the contents of the glands disperse if added to an excess of water, this never happens once some air drying has taken place; one must therefore assume that some denaturation of protein has occurred through the desiccation process. The process is apparently speeded by such chemical denaturants as ethyl alcohol or phosphomolybdic acid, but not merely by pure oxygen. From the biological view point, it is much more important to note that desiccation leads to the formation of a spongy material; when injected with cobalt naphthenate (Wigglesworth, 1950), the presence of air spaces throughout the sponge is readily demonstrated and the covering of cement over the egg-shell does not therefore prevent the transfer of air in gas phase to the surface of the chorion. From the point of view of penetration of poisonous materials, it must equally be remembered that the cement will have no real effect in preventing liquids from reaching the chorion surface, provided they are capable of wetting the surface of the cavities in the material. Further, the cement is hygroscopic, and (see later) thus maintains a high water concentration immediately outside the shell. It was observed that failure to hatch when eggs were kept in low humidities was not associated with cessation of embryonic development, but with a very hard brittle shell, and must therefore be due to the inability of the embryo to penetrate.

Chemical properties of the cement.

From staining reactions with safranin, fuchsin, orange G, together with xanthoproteic, ninhydrin, etc., tests (Beament, 1951) both matrix and granules are acidophilic protein; the argentaffin test indicated the presence of some reducing material. The cement however, not only reduces osmic acid, but is also stained very slightly by the Sudan fat stains; corrosive materials such as strong nitric acid, or sodium hypochlorite, break down the cement, releasing oily droplets, which might indicate that the cement is a lipoprotein. But solvents, such as chloroform and ether will remove the lipid part of the cement and the extracted material has a strong reducing action. It is obviously, therefore, an unsaturated lipid, not chemically attached to the protein part of the cement, and bringing the material into line with those already described for *Metatetranychus* (Beament, 1951). As with the cement of that mite, this material is attacked by strong formic acid, and by strong sulphuric acid—a property not common to most insect shell components. Treatment with lipid solvents does not cause any marked visual change in the cement, but it does lose its adhesive property to a large extent, and becomes much more brittle on drying. It also shows much more intense staining reactions with aqueous reagents; thus this small amount of lipid is probably of considerable importance in regulating the rate of water absorption by the cement, and may well affect the penetration of such liquids through the respiratory spaces in it. For ovicidal experiments, the eggs were treated with acetone, which would presumably act on the lipid component and make the cement less adhesive.

The cement therefore conforms to the pattern of the “permanently adhesive”

type *Metacltranychus*, *Aphis*, etc. (Beament, unpublished work) and not to the hard type of *Rhodnius*. The cement of *Diatraxia oleracea* (L.) (Salkeld & Potter, 1953) undoubtedly contains very much lipid material. The cement of *Pieris* has the additional property however, of containing a water-soluble yellow compound, apparently a strong reducing material attached to a protein, which can diffuse into gelatin and colour it. It is suggested that this may be a phenolic material, though it does not appear to be of any great importance in hardening the cement.

The chorion.

The chorion of the ovarian, and of the newly laid eggs, is very soft and plastic; it will remain so, provided it is kept in contact with liquid water, but exposure to low humidities, or heating to a temperature of 55°C. produces some irreversible hardening: this probably occurs in nature due to normal exposure. Nevertheless, the pliability of the shell is directly related to its water content, and does not seem to be altered by such things as extraction with chloroform. The chorion appears to be composed of two layers (fig. 1, D) which could not be separated mechanically or chemically. Staining reactions with safranin, orange G., picric acid, iodine, together with xanthoproteic and ninhydrin tests show that both layers are basically of protein. The shell layers do not stain with the Sudan fat stains, or with osmic acid; there is no liberation of fatty droplets when either layer is dissolved by strong acids, etc., and staining reactions are not intensified by chloroform extraction. One therefore concludes that no free, or chemically incorporated lipid exists in either chorion layer. The inner layer only gives an argentaffin reaction, but this is most pronounced in the granular material (compare *Rhodnius*: Beament, 1946a). It also colours strongly with *p*-benzoquinone, and would appear to be of partially quinone tanned protein. It is perhaps a little surprising that the outer layer, which is apparently untanned, will not take up the quinone. However, the outer layer dissolves more readily in strong acids, etc., and must be regarded as less cross-linked. When strong nitric acid acts on the shell, it breaks down in the cold, giving rise to small granules which dissolve eventually after some 24-48 hours. The whole of the veins and sculpturing disappear in this process, and must be regarded as formed from the outer chorionic layer, though when more slowly acting materials such as strong ammonia are used, the veins are not attacked. They are obviously of more resistant material. Urea, strong formic acid, pepsin or trichloroacetic acid are without effect on either layer; trypsin on the other hand digests the outer layer and veins only. This particularly confirms the suggestion that the inner layer is of tanned material, while the outer is free of such linkages. The action both of lithium iodide and of sodium thioglycolate was tried on the outer and inner layers, but did not materially affect their resistance to solution. It seems very unlikely that sulphur linkage plays any great part in their construction. Since both layers stain with water-soluble materials, even when whole eggs are dipped in the solution, there cannot be any important property in either layer preventing quite big water-soluble molecules from passing through them to the inner surface of the chorion.

Comparing this egg with that of *D. oleracea*, for which Salkeld & Potter (1953) have carried out a similar piece of work, it will be seen that the gross morphology of the shell is similar, but we have been unable to find any layer corresponding to the thin exochorion of lipoprotein with which *D. oleracea* eggs are covered.

The lipid layer.

Between the inner surface of the chorion and the outer embryonic layer lies the lipid layer (fig. 1, D). It is present in ovarian eggs, and can be obtained by extraction, or as droplets following the breakdown of shells from which the embryonic contents have been washed. It is important to note that this material is a freely mobile oily substance, and not a hard wax, as has been previously found

in insect eggs in this position (*Rhodnius*, *Melanoplus*, *Metatetranychus* (a mite), etc.: Beament, 1946b; Slifer, 1946). A similar material has been noted in eggs of *Psylla mali* (Schm.) (Beament, unpublished work). After extraction with chloroform, the oily residue blackens strongly with osmic acid, and decolorises iodine solutions. It is obviously an unsaturated material, and this again is unique so far as our present knowledge of apparently waterproofing lipid materials is concerned. It will be shown later that this layer constitutes the chief barrier to the penetration of water-soluble poisons into the mature egg.

An attempt was made to determine the "transition temperature" of the egg, i.e., the temperature at which its lipid layer becomes markedly more permeable to water. For this purpose, eggs were placed in a solution of sodium chloride, saturated at room temperature (Beament, 1951), for short periods of time at specific temperatures; the transition temperature is given when the egg rapidly collapses, losing water by osmosis. The results are given in Table I.

TABLE I.
Transition temperatures of the egg in degrees C.

Age of eggs	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
24 hours ..	59-60	60-61	63-64	62-63	63-64	63-64.5	60-61
48 " ..	63-64	63-65	64-65	65-67	67-68	68-70	66-67
72 " ..	65-68	67-69	68-70	68-69	69-71	70-71	69-70
96 " ..	64-67	69-70	70-71	68-69	70-72	71-73	71.5-72.5
Above 96 hrs. and ready to hatch ..	67-69	69-70	69-70	69-70	70-71	71-72	71-73

Transition temperature of batches of unfertilised eggs from the ovary are
64, 63-64, 64-65, 62-63°C.

The transition temperature is not particularly sharp, and is apparently appreciably different from one egg batch to the next. This is certainly not a feature of the *Rhodnius* egg (Judge, 1953) which has probably been most carefully measured. There is also a significant increase in the transition point as the egg ages. This may amount to as much as 7-13 Centigrade degrees over the four-five days of embryonic development. It is rather remarkable also that a grease, which immediately calls to mind that on the cockroach (t.p. 33°C.) has apparently a transition temperature around 60°C.—which one would associate with a hard waxy material. It could be suggested that the properties of unsaturated materials as waterproofing agents may be very different from those of the saturated lipids. It could also be put forward that the gradual increase may be due to some loss of a more volatile component from the layer, but both ideas would need much further work to elucidate them. Salkeld & Potter (1953) have shown a similar phenomenon in the transition point of eggs of *D. oleracea* though they did not suggest that the changes were significant. (Dr. Potter has been good enough to confirm that his figures are in Centigrade degrees, and not Fahrenheit, as shown in his paper.) These authors regarded the material in *D. oleracea* as being a hard wax.

In hypotonic solutions, of course, eggs will swell, the speed depending on the permeability of the surrounding membranes for a given strength of solution.

Accordingly, eggs were kept at various temperatures in 0.1 per cent. basic fuchsin. Below 49°C., there is no swelling in 24 hours. Above this temperature there was a progressive increase in swelling and in the staining of the embryo, so that at 56°C., the embryo became quite stained in 24 hours, while above 60°C., the process was rapid and the eggs burst. This information is a necessary confirmation of the high transition temperature of the unsaturated greasy material. Its importance to penetration of toxic materials will be discussed later.

Changes in the egg-shell after oviposition.

In general, the outer chorionic layer appears to undergo little change after laying, but the inner tanned layer of eggs inside the female is readily soluble in strong ammonia and 10 per cent. potassium hydroxide. It would appear that much of the tanning of the inner chorion goes on subsequent to fertilisation, but is complete within the first day of embryonic development.

Embryonic coverings.

We have so far concluded that there are very few post-fertilisation changes in the chorion or cement coverings of the egg; equally the lipid layer will not apparently change greatly in characteristics during embryonic development. It is therefore of some importance to investigate the nature of any extra-embryonic membranes interior to the lipid layer and to discover whether any changes in them may run parallel with changes in the oviducal resistance of this egg. It is already apparent that only the lipid layer of the chorion will give resistance to the penetration of aqueous materials, while there would appear to be little protection against oily ones.

The egg, before laying, is surrounded by a typical vitelline membrane, which appears to follow the usual pattern of transformation into a fertilisation membrane (fig. 1, C). This layer is composed of proteinaceous material—it shows no staining reactions for fatty materials, nor are any produced when it is broken down. It would appear to be readily permeable, not only by water but to comparatively large water-soluble molecules of dye. But within 24 hours of laying, the inner part of the vitelline membrane has disappeared, and is progressively replaced by an epembryonic membrane (see Beament, 1949). As this occurs, it becomes very resistant indeed to chemical attack. For example, it takes 48 hours for complete solution to occur, when the epembryonic membrane of 24–72-hour-old eggs are placed in 5–7 per cent. sodium hypochlorite, in strong sulphuric and hydrochloric acids or 10 per cent. potassium hydroxide in the cold. The structure is rather more soluble in strong nitric acid, alone or with potassium chlorate. The chemical nature of this material is obviously very similar to that of the corresponding membrane in *Rhodnius* (Beament, 1949), and the shell layer of eggs of *Metatetranychus* (Beament, 1951).

But it is very important to note that when the embryo approaches four days old, the resistance of the membrane certainly falls very appreciably, presumably due to chemical action of the living material softening it; it does not, however, revert entirely to the properties of the original vitelline membrane, but leaves room for supposing that the membrane at this stage corresponds to the fertilisation membrane.

To elucidate these happenings further, a series of experiments, using the effect of pre-treatment with trichloroacetic acid and sodium thioglycolate on the action of trypsin, were undertaken. Up to two hours after laying, the vitelline membrane and associated fertilisation membrane are dissolved by trichloroacetic acid in 24 hours. Solution is not complete if the egg is allowed to develop for four hours, and there is apparently no attack on the epembryonic membranes at all from that stage of development until 96 hours is reached. From then until hatching, the membranes are again dissolved by trichloroacetic acid, thus showing the preparation

for hatching carried out on the membranes by the embryo. Similarly, trypsin at pH 8.5-8.8 and 37°C. breaks down the early and late membranes, but not in the intermediate period. Now if membranes in the more resistant stage are treated first with sodium thioglycolate at 30°C. at pH 12, they are not dissolved, but following this treatment they are readily broken down by both trypsin and by trichloroacetic acid. Similarly, concentrated lithium iodide solution will render the membranes of 24-hour-old eggs soluble in trichloroacetic acid (though much more slowly) but will not enable trypsin to break them down. Had the resistance of these membranes been due merely to the typical cystine linkages, such as are found in keratin, the whole membrane should have been dissolved in sodium thioglycolate; had the resistance been similar to silk, it should have been soluble in lithium iodide. It therefore seems correct to conclude that the form of linkage in this membrane—which can be formed and later broken by the embryo—is similar to a cystine link, but not identical, and that there is some residual bond, attacked by trichloroacetic acid, which remains after some form of devulcanisation has occurred. The authors are grateful to Dr. M. G. M. Pryor for the suggestion that the actual linkage in this membrane may be a form of thioquinone, which might be expected to behave in this way.

Relation of embryonic membranes with the micropyle.

Beament (1949) has pointed out the extreme importance of studying the form of the micropylar and respiratory structures of insect egg-shells, for they may provide the most important sites of entry of toxic materials, and give good reasons for the pattern of events following ovicidal treatment. In the egg of *Pieris*, one can appreciate the problem produced by the oil layer, for sperm could not in all probability successfully move through it. It is in fact discovered that the ovarian egg, and the egg immediately after laying, has its inner membranes applied closely and quite securely to the inner lip of the micropyle (see fig. 1, E). The oil layer is not, therefore, complete round the egg, and sperm can penetrate from the micropyle directly into the surface of the vitelline membrane. But within two hours after fertilisation, some form of movement, which might be regarded as a slight shrinkage of the egg-shell, takes place—the junction between micropyle and membrane breaks, and the oil flows across, thus becoming a complete layer, and sealing the micropylar opening (fig. 1, F). This activity probably has considerable bearing, both on the resistance of the egg to penetration of ovicides, and on the rate of water loss through desiccation.

Water relations of the egg.

Investigation of rates of water uptake and loss from the egg under various conditions were undertaken to discover whether the egg has any active water uptaking mechanism during embryonic development, whether there was any evidence for a further waterproofing mechanism produced in the embryonic layers, the amount of water which could be lost before lethal effects were produced, the importance of the cement in waterproofing, and the humidity range in which the egg could successfully develop.

(a) *Water content.*—The fertilised egg at oviposition (with cement) weighs approximately 0.22 mgm. Of this, 0.018 mgm. is cement and 0.009 mgm. shell; the egg contains some 85 per cent. by weight water, while the newly hatched larva contains 81 per cent. It will be noticed that the cement accounts for nearly eight per cent. of the total weight of a newly laid egg. It therefore occurred that its properties might be independently investigated so far as water uptake and loss were concerned, thus enabling a more satisfactory assessment of water relations of the underlying material. Similar experiments were carried out on vacated shells.

(b) *Hygroscopicity of cement and chorion.*—Cement was obtained in quantity

from the appropriate glands of mature females. It contains over 60 per cent. by weight of water, most of which is readily lost into room air, but a residual amount that can only be extracted in a desiccator. About a quarter of the maximal water content can be regained in saturated air in two hours, while all the water lost by drying can be taken up again under these circumstances in 24 hours. The unfertilised egg taken from the calyx has no cement on it, and after drying it does not regain any measurable amount of water in saturated air, whereas the fertilised egg, so dried for 24 hours, regains at 100 per cent. relative humidity, exactly the amount of water which would be accounted for by the amount of hygroscopic cement on its surface. This result was the same, regardless of the age of the fertilised egg, and whether it was alive, or had been killed by cyanide fumigation. It therefore can be concluded that this egg has no active or passive means of taking up water from the atmosphere, other than a direct equilibrium between atmospheric humidity and the cement. It seems most unlikely that this water is in any way used by the embryo, but the water content of the cement (for both it and the chorion become much more rigid with drying) may materially affect the success of eclosion. The chorion must obviously take up some water in softening but the amount was not measurable.

(c) "*Active*" *water retention mechanism*.—Unfertilised eggs lose water rapidly on desiccation (Table II)—the initial rate of loss is of the order of 4 per cent. by weight per hour, as compared with fertilised eggs which lose about 0.5 per cent. by weight per hour under similar conditions. After 48 hours an unfertilised egg has lost almost the entire water-content whereas a fertilised egg is still developing,

TABLE II.

Loss of water in dry air (25°C.) from living eggs, or eggs killed by fumigation with hydrogen sulphide for half an hour.

Age of eggs							Live eggs	
	Percentage loss after :						Per cent.	
	4 hours		8 hours		24 hours		Reached 96 hr. stage	Hatched
	Alive	Dead	Alive	Dead	Alive	Dead		
Less than 4 hours ..	4.9	5.9	8.9	13.01	22.8	25.4	8.7	0
24 hours ..	2.9	8.06	7.2	12.5	17.8	26.7	35.7	0.8
48 „ ..	3.9	7.5	6.7	12.8	18.5	26.7	48	0
72 „ ..	3.5	6.02	10.0	14.4	26.5	38.5	74	16
96 „ ..	8.9	3.01	20.8	12.04	41.1	33.7	—	76
Unfertilised eggs from ovary ..	18.4		38.5		54.7			

having lost about 30 per cent. of its total water. On the other hand, there is no significant difference between the rates of loss of normally developing eggs, as compared with those killed by hydrogen sulphide within four hours of laying, under similar conditions of desiccation. It would seem very unlikely that death produces any "breakdown" in a waterproofing mechanism, but the process of fertilisation

is accompanied by some irreversible improvement in waterproofing. One would like to suggest that this increased improvement is associated with the withdrawal of the egg-content from the micropyle, thus completing the oil seal around the egg (see p. 115, above), but the evidence must remain somewhat inconclusive on this point. The experiments cited above on cement must exclude the cement covering from accounting for this additional waterproofedness—if anything, the existence of such a porous covering would increase evaporation rates from the shell surface.

(d) *Changes in waterproofing during development.*—Eggs of various stages of embryonic development were killed with hydrogen sulphide vapour and the rate of water loss in 0 per cent. relative humidity determined. The results certainly do not indicate that there is any additional waterproofing mechanism added during development (compare *Rhodnius*: Beament, 1949). There would appear to be a gradual increase in permeability as the egg ages, such that at 72 hours old (Table II), the shell appears to be rather more than 10 per cent. more permeable; there is equally no appreciable increase in water loss during the solution of the epembryonic membrane by the embryo, immediately prior to hatching. It must be remembered that the availability of water from the interior of the egg will change as development proceeds, so that one should only deduce from these figures the salient fact that the lipid layer inside the chorion is apparently the sole important water barrier, and that its efficiency is not markedly impaired throughout the life of the egg. It is possible that it requires stabilisation by the production of the fertilisation membrane, for the unfertilised egg is much more permeable—it is certainly not destroyed by the embryo immediately before eclosion.

(e) *Effect of water loss on survival.*—The experiments outlined above were carried out on large numbers of eggs—often on batches of 300 or more per experimental determination. In every case where desiccation experiments were started with living eggs (Table II) notes were taken on the percentages of larvae which completed development to the stage where the black head could be seen through the chorion—about 96 hours old. Note was also taken of the numbers of larvae which hatched from these eggs, at the particular humidity, and some indication was obtained of the amount of water loss of eggs from which hatching was successful, as compared with those where death occurred, either before or after completion of embryonic development. The following information seems worthy of comment. Eggs kept at 100 per cent. R.H. do not lose a measurable amount of water during development. On the leaf we would suppose eggs to be in an almost saturated environment and at least judging visually, there is not an obvious shrinkage during development. Yet, one-third of eggs placed 24 hours after laying in 0 per cent. R.H. for 48 hours, whence they lose 30 per cent. of their water, still attain full development. In a further experiment, where desiccation was continued for four days, again about one-third completed development; here the rate of water loss was appreciably slower in the portion completing development, but one larva out of an original batch of 125 eggs hatched. When, however, eggs were desiccated immediately after laying, less than 10 per cent. of the eggs completed development and none hatched. When experiments were started with older eggs, the proportion which completed development also increases significantly, but even where desiccation was carried out on eggs within 24 hours of hatching, a quarter of the larvae did not hatch (Table II). Yet where eggs immediately on laying were placed in higher humidities, even though their water loss was appreciable (up to nearly 40% in some cases) eclosion rates were very high (Table III)—and would probably have proved almost 100 per cent. had not some of the material been eaten by emerging larvae.

A high humidity, and presumably therefore a softened shell, is obviously an essential to the hatching process, much more than a high internal water-content. It seemed more likely to the authors that eggs which survived the desiccation experiments were merely those with a more efficient form of the waterproofing

mechanism, since in randomised experiments there was no significant difference between rates of water loss for large batches of living and of dead eggs. One must remember here that in its natural environment, the egg has not apparently great need of an efficient water conservation mechanism, and it was obvious that considerable variation occurred even among eggs of one batch from one female.

TABLE III.

Percentage successful development and successful hatching of eggs at 25°C. in different humidities.

Age of eggs	30% R.H.		70% R.H.		90% R.H.		100% R.H.	
	Reached 96 hr. stage	Hatched	Reached 96 hr. stage	Hatched	Reached 96 hr. stage	Hatched	Reached 96 hr. stage	Hatched
Less than 4 hours ..	—	—	16	61	1	70	0	77
24 hours ..	44	26	—	—	—	—	2	73

At 70% R.H. only 6% eggs were eaten by the larvae, at 90% about 28%, and at 100% R.H. about 23% eggs were eaten by them.

Oxygen relations of the egg.

The egg has a typical series of respiratory pores, concentrated towards the front end, running through the chorion (fig. 1, D), as revealed by injection with cobalt naphthenate (see Wigglesworth & Beament, 1950). The respiratory system of *D. oleracea* (Salkeld & Potter, 1953) is even more concentrated into a band round the anterior end of the egg. The porous cement would not in any way interfere with access of oxygen to the system. But in ovidical experiments it is important to ensure that any dipping process does not block, or interfere with the oxygen supply of an egg (see p. 119). Immersion for 24 hours, where the surface of an aqueous solution is exposed to the atmosphere certainly would not affect an ovidical result through interfering with oxygen; eggs 24 hours old will successfully develop in aerated distilled water in a sealed vessel, allowing about 1 cc. water to each egg, though of course they will not hatch. Most ovidical experiments involved very much shorter exposures than one hour, and the controls for longer periods did not indicate any interference with respiration.

To show that the effect of immersion is purely one of restriction in oxygen, eggs were confined in various volumes of distilled water, either sealed, or with different areas of the water surface exposed for gaseous exchange—they were also sealed in 5 volume hydrogen peroxide and incubated in water through which oxygen was bubbled continuously. In every experiment there was apparently normal development if there was sufficient oxygen, even when immersed for three or four days. It is also not without interest that eggs were successfully incubated in atmospheres containing more than 50 per cent. oxygen. Many of the larvae in these experiments died soon after hatching.

It was not possible to show any continuous spongy air layer in the inner regions of the shell, at the base of the respiratory pores (compare *Bombyx* and *Ephesia*: Wigglesworth & Beament, 1950). These appeared to lead straight to the oily waterproofing layer, and it might be suggested that this layer, which occupies a position where one would have expected to find a sponge, is itself acting as the distributing medium, for oxygen is comparatively soluble in such a material.

But the need for the respiratory pores to be freely open was shown by the lethal effect of heavy oils. P. 31 oil, and petroleum jelly are both extremely

poisonous; if the front ends of eggs are merely touched against these materials there is a complete kill. Some eggs, however, do survive having their rear ends applied to P. 31 and vaseline, and we must conclude that they do not spread so efficiently that they will reach the front region and so block the pores of all eggs. The effect of dipping in P. 31 for half a minute can be removed completely by repeated washing with petroleum ether and with acetone; the oil is entirely superficial.

Similarly, it was shown that solutions containing wetting agents, such as Teepol, can be lethal, if eggs are immersed in them for several hours, as compared with similar volumes of pure water. One would not imagine oxygen diffusion through either to differ, but Teepol does wet the egg and probably therefore displaces, slowly, the air from the cement which one might imagine as acting as a physical gill.

Ovicidal Experiments.

Introduction and methods.

It is well to point out some of the difficulties encountered as well as the methods used in the ovicidal experiments. For example, very misleading results are obtained, unless eggs are incubated in a water-saturated atmosphere before and after ovicidal treatment. We have previously shown that eggs can survive a remarkably high water loss and complete their development, though they usually fail to hatch; there is also a considerable variation in waterproofedness. If such a variation had been accompanied by a similar variation in ovicidal resistance, this would presumably have shown in the experiments as an increased efficiency of kill, when eggs are incubated at lower humidities. But the effect of low humidity is obviously very much more complex and we have been unable to relate it to any aspect of the ovicidal picture.

The surface of the chorion of the egg is strongly hydrofuge, but, of course, after laying it is covered by cement. This material has similar contact properties to tanned protein—it is difficult to wet when dry, but readily wetted when saturated with water—and since it has been shown that the cement absorbs water in a saturated atmosphere, little difficulty in wetting the surface during ovicidal experiments was anticipated. But this proved not to be the case; the spongy cement retains a mantle of air (which probably acts as a physical gill during immersion) and in all experiments, the eggs were forcibly immersed in the ovicidal liquid using a camel-hair brush. It is unlikely that this air is ever replaced by water even if eggs are immersed for many hours, where there is a suitable aeration of the fluid. Thus in typical experiments, where immersion was for five minutes, it is most doubtful if the aqueous liquid gets into this porous system. On the other hand, displacement of air by oily materials is likely to be very rapid, and we have already shown that they prove lethal, through a purely smothering action, unless they are sufficiently volatile to disperse from the shell before asphyxiation sets in.

It has already been demonstrated that, after lipid extraction has removed or disrupted the lipid layer, quite large water-soluble molecules of stain can penetrate to the embryo over its entire surface—whereas in the untreated egg only immediately before or after fertilisation is there any penetration of stain, and that, entirely through the micropyle and area of the embryonic coverings applied to it. It would probably be correct to conclude that, in our experiments, penetration of aqueous materials takes place through the material of the cement, and through the substance of the shell, rather than by displacement of air from the cement sponge and respiratory pores of the chorion. Lipophilic materials, on the other hand, would be expected to pass through the respiratory system into the lipid layer, and therefore have a much more efficient path of entry, apart from the possibility of their acting purely physically should they not be volatile.

Eggs were removed from the leaves using acetone to soften the cement. This

has no harmful effect provided immersion is only sufficient to soften the adhesive. They are extremely delicate, and were handled with a camel-hair brush. All experiments, and subsequent incubation were carried out at 25°C. and 100 per cent. relative humidity.

(a) *Water-soluble salts of heavy metals.*—Metal salts used were the chlorides and acetates of cobalt, copper, manganese, and nickel. No harmful effect on eggs 24 hours old or more was produced by dipping for five minutes in cobalt, manganese and nickel salts, used in concentrations up to 1 per cent. by weight in water. Post-hatching casualties among larvae could be attributed to the stomach poison action of materials they obtain when eating their shells; there can therefore be no doubt of a reasonable residue of poison in the substance of the shell and cement. These materials did not become any more efficient either when used in combination with strong wetting agents (1 per cent. Teepol or 0.1 per cent. C.09993, a polyethylene glycolester) or when immersed for five minutes following evacuation, so that the liquid is forced into any air spaces in the shell (Beament, 1949). It is quite obvious that these electrovalent and purely water-soluble materials, with no lipid solubility, are not getting to the living material; potentially they are obviously very toxic. But eggs less than six hours old are quite susceptible to, for example, 1 per cent. nickel chloride solutions, especially in a wetting agent. The emulsifier itself has no harmful effects, and controls, using solutions of glucose, sodium chloride, etc., eliminated the possibility of these strong solutions having an adverse effect through causing exosmosis, during the immersion period of any experiment. Again, 1 per cent. nickel chloride does not become toxic until older eggs are immersed for six hours with a wetting agent, or twelve hours alone (Table IV)—penetration in the older eggs is obviously very slow indeed. It is

TABLE IV.

Percentage kill of 24-hour-old eggs following 6 and 12 hours in 1.0 per cent. nickel chloride or nickel acetate.

Treatment	Kill (%) after	
	6 hours	12 hours
Nickel chloride alone	20	80
Nickel chloride in 1% Teepol	100	100
Nickel acetate alone	20	40
Nickel acetate in 1% Teepol	80	80
Teepol 1% alone	40	70
Water alone	20	20

There is 10 per cent. mortality of the untreated eggs.

important, however, to point out that copper acetate has a slightly more toxic action, when compared with copper chloride or with the acetates and chlorides of the other metals mentioned above; it has the significant property of being soluble in ether as compared with the others.

In many of these experiments it was noticed that eggs, which may have been treated at 24 hours old, completed development, but died just before hatching, or in the hatching process. It would be difficult to decide whether this was due to the embryo releasing material in the epembryonic membrane, or in the chorion, having penetrated it; this will be discussed later.

(b) *Oil-soluble materials.*—Dichlorodiphenyl-trichloroethane (DDT), as may be expected, is not toxic, even when used at 0.1 per cent. for five minutes in acetone, although almost all experiments ended with larvae dying immediately after hatching. Acetone, ethyl alcohol and petroleum ether are not toxic for

short periods of immersion, although chloroform is completely toxic at all stages. Dinitro-o-cresol was used in a series of experiments (Table V). In a 0.1 per cent. solution in petroleum ether, one minute's immersion proves completely toxic to eggs from laying to 24 hours old—and the poison so obtained cannot be removed by immediately washing the eggs rapidly through several changes of the solvent. At 0.01 per cent., rapid washing will remove the poison, and eggs will survive.

TABLE V.

Percentage kill of eggs following one minute in a solution of dinitro-o-cresol in petroleum ether.

Concentration of DNOC	Age of eggs	Kill (%)	
		Washed with more solvent after treatment	Unwashed
0.01	Less than 6 hours	0	100
0.1	"	100	100
0.01	24 hours	0	100
0.1	"	100	100
0.01	48 hours	10	100
0.1	"	0	100
0.01	72 hours	0	80
0.1	"	0	100
0.01	96 hours	0	100
0.1	"	100	100

Untreated eggs or those treated with petroleum ether alone showed no mortality.

There is no significant difference between the susceptibility of eggs before or after an age of 6 hours. Eggs 48 hours old, or more, would appear to be slightly more resistant.

When used in aqueous solution, pH 7, a concentration of 0.07 per cent. (used as a saturated solution) appeared to be toxic to all stages when immersed for one minute. Experiments were performed, using a constant product of strength and time of immersion, varying from 0.07 per cent. for one minute, to 0.0035 per cent. for twenty minutes, and similar combinations (Table VI). The results show that

TABLE VI.

Concentration X time/kill for aqueous solution of dinitro-o-cresol for eggs 24 and 48 hours old.

Treatment with DNOC	Kill (%)	
	24-hr. eggs	48-hr. eggs
0.07% for 1 minute	100	100
0.035% for 2 minutes	100	60
0.014% for 5 minutes	90	100
0.007% for 10 minutes	50	0
0.0035% for 20 minutes	0	20
Water only	0	0
No treatment	0	0

again, eggs of 48 hours and older are somewhat more resistant; the most dilute solutions for a correspondingly longer immersion are not as effective to any stage. One must not forget in these experiments that DNOC can act as a fumigant, and that a residue in the shell may be acting in this way—it would have longer to act where a young egg is dipped, for this takes a longer time before the endpoint of hatching is reached. This point has been verified in later experiments.

(c) *Fumigation experiments*.—Where eggs are kept in a vessel containing a known amount of DNOC, again, younger eggs are slightly more susceptible, but there is no sign of embryonic development in these eggs, and it seems very doubtful if this great toxicity can be explained on other grounds, if the period of fumigation is kept constant.

All stages of the egg appear similarly susceptible to mercury vapour, but 12–24 hours exposure is necessary to kill; hydrogen cyanide penetrates slowly into older eggs but hydrogen sulphide is rapidly fatal at all stages.

TABLE VII.

Percentage kill of eggs following 5 minutes in solutions of mercuric chloride and of other materials containing equivalent concentrations of mercury ions.

Treatment and concentration of salts (%)			Kill of eggs aged	
			Equivalent concentration of mercuric chloride (%)	24 hrs. 48 hrs.
Mercuric chloride	1.0			100 100
" "	0.5			100 90
" "	0.1			100 40
" "	0.05			70 20
" "	0.025			— —
" "	0.01			0 20
Mercuric acetate	2.4	2.0		10 0
" "	1.2	1.0		20 10
" "	0.6	0.5		20 0
" "	0.12	0.1		0 0
Mercuric bromide	0.61	0.462		100 100
" "	0.305	0.231		100 80
" "	0.132	0.1		100 100
" "	0.066	0.05		100 100
" "	0.033	0.025		0 0
Mercuric nitrate	0.6	0.5		60 —
" "	0.3	0.25		0 —
" "	0.12	0.1		0 —
Arsenic trichloride	0.33	0.5		70 —
" "	0.165	0.25		60 —
" "	0.066	0.1		30 —
" "	0.033	0.05		50 —
Arsenic trioxide (pH 10 or more)	0.355	0.5		100 —
" "	0.177	0.25		100 —
Arsenic trioxide (pH 6–7)	0.355	0.5		0 —
" "	0.177	0.25		0 —
Lithium chloride	0.5	3.2		0 —
" "	0.078	0.5		0 —
Water only		—		0 0
No treatment		—		0 0

(d) *Materials with oil and water solubility*.—In view of the slight increased toxicity of cupric acetate over similar compounds without a degree of lipid solubility, and the greater effectiveness of the oil-soluble compounds—a series of experiments was devised to compare mercuric chloride and bromide (covalent water- and lipid-soluble compounds), mercuric acetate (electrovalent water-soluble compound), mercuric nitrate, arsenic chloride (electrovalent water- and lipid-soluble compounds) and lithium chloride (strongly electrovalent but with lipid solubility (Table VII)). From these experiments, it would appear that mercuric chloride is a highly toxic material as used in this work. The egg, immediately after laying, is appreciably more susceptible, and there is also a slight increase in resistance at the 48-hour stage. Mercuric bromide is also extremely efficient as an ovicide if used at concentrations containing a corresponding number of mercury ions. When compared at equal strengths of metal in solution, mercuric nitrate is about one-tenth as poisonous as the chloride, the ratio being fairly consistent regardless of the age of egg. Arsenic chloride is similar in its action, though surprisingly very effective against very young eggs. It is particularly interesting to note that equivalent solutions of arsenic oxide at pH 6–7 are almost non-toxic; the entire effect of this material in alkaline solution (pH 10) can be attributed to the action of the alkali. Lithium chloride is not toxic.

Conclusions.

In comparison with eggs of *Diatraea oleracea* (Salkeld & Potter, 1953) we have also found that the cement and main shell layers of eggs of *P. brassicae* are not effective in preventing the entry of water-soluble poisons. Likewise, there is no evidence that the micropyle forms a preferential site of entry. It is clear, however, that increased resistance, due to the formation of epembryonic membranes, and subsequent decrease with their solution, is very much more pronounced in eggs of *D. oleracea*. But, on the other hand, there is no reason to suppose that the very young egg of the latter species is as highly susceptible.

Our conclusions accord well with the general pattern of ovidical entry outlined by Beament (1948, 1949, 1951), but in this egg almost the entire resistance seems to be concentrated in the unusual lipid layer. This may certainly be associated with the comparatively simple shell, the short life of the egg stage and the high humidity in which it naturally develops.

Summary.

The structure of the shell of eggs of *Pieris brassicae* (L.), together with changes in it and associated membranes during embryonic development, have been investigated in relation to the penetration and toxicity of simple chemicals. The rigid outer shell consists of two proteinaceous layers, covered externally by a relatively hydrofuge cement, by which the egg is attached to the leaf surface. The egg has respiratory pores over its surface, and a single apical micropyle penetrating these layers. The inside of the rigid shell is lined with a layer of unsaturated oil—an unusual feature for an insect egg. When the egg is first laid, the vitelline membrane is directly applied to the inner surface of the solid shell over the region immediately around the micropyle, but within four hours this contact is broken, and the oil layer flows into this region also, and becomes complete. As development proceeds, the vitelline layer is replaced by membranes of embryonic origin, but before eclosion both these epembryonic layers, and also the oil, are resorbed.

The egg is remarkably resistant to water-soluble poisons which have no oil-solubility, except during the first four hours of development. This resistance is attributed almost entirely to the oil layer, and the early susceptibility to its absence over the micropylar region. These changes are not reflected in the effect of oil-soluble poisons or fumigants. The solid portions of the shell do not seem

to be of great importance in restricting the entry of liquid poisons, even though the cement is comparatively hydrofuge; from experiments with wetting agents and with eggs immersed in poisons under vacuum, it does not appear that the respiratory air spaces in the shell are preferential channels of access; rather, the poisons penetrate through the solid portions of the shell. This penetration, even of oil-soluble materials, is slow, for they can be effectively washed out of the shell again, some considerable time after dipping. On the other hand, non-volatile oily materials can interfere with the respiration of the egg by blocking the air spaces in the shell.

The secretion of epembryonic layers does not appear to change the resistance of the egg to water-soluble materials; this is to be expected, for they do not contain lipid. On the other hand they do add appreciably to the resistance to oil-soluble materials. There is no evidence that poisons are accumulated in these epembryonic membranes, and released during the pre-eclosion period. Experiments with covalent compounds, such as mercuric chloride, suggest that their oil-solubility accounts for their toxicity, whereas electrovalent compounds containing similar heavy metals are only effective while the direct micropylar path of entry is available to them.

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BIOLOGY AND ECOLOGY OF THE GARDEN CHAFER, *PHYLLOPERTHA HORTICOLA* (L.). III.—THE GROWTH OF THE LARVA.

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In nature, the eggs of the Garden Chafer, *Phyllopertha horticola* (L.), are laid about $1\frac{1}{2}$ inches deep in the soil in June and hatch about 14th July. The first moult occurs about 2nd August, the second moult about 3rd September. The third-instar larvae stop feeding and go into hibernation between the end of October and the beginning of December. Thus the whole feeding period lasts about 110 days, the larva spending about 20 days in the first, 30 days in the second and feeding for a further 60 days of the third instar. When it stops feeding the grub empties the gut, burrows down to a depth of about 2 inches and presses out a little cell in the soil. Here, in a quiescent state, it spends the winter and here it pupates, emerging from the soil only when an adult beetle.

The above account is summarised from Part II of this series (Milne, 1956). The dates and figures are means from five years of observation in the Lake District. There is considerable variation from year to year, from place to place and within each population.

It has been shown (Raw, 1951; Milne & Laughlin, 1956) that the egg production of the female chafer depends almost entirely on the weight of the hibernating larva or the pupa, that is, on the amount of reserves stored in the fat body. The potential energy stored during the 3-4 months of feeding is sufficient for the completion of the reproductive cycle; the adult does not need to feed at all in order to mate and lay the usual quota of eggs. Thus the growth of the larva and its variation under different conditions play an important part in determining the reproductive rate of the population.

It must be emphasised here that the reproductive rate of a population is a resultant of the environment, in all its complexity, acting on the physiological and behavioural properties of the species. This paper is limited to a discussion of the effect of some environmental factors on one of these properties, namely the ability of the larva to grow and put by stores of material for use in reproduction. In one or two places the limit is overstepped to include interesting mechanisms unexpectedly revealed by the work described. For example, the sex ratio of populations of pupae varies quite widely in nature (see Milne, 1956) and it was found that, under certain conditions, mortality selective of female larvae could arise at the end of the larval feeding period (but see p. 152).

The Feeding Period.

The newly hatched larva weighs about 3 mg., the fully fed, hibernating grub about 200 mg. The five experiments described below show how the weight of the larva changes between these two points.

Growth at constant temperature (Experiments 1 and 2).

McColloch (1917) fed the larvae of various American Scarabeids on germinating seeds in fine damp soil in salve boxes with tightly fitting lids. The same method was used for the Garden Chafer except that the containers were made of plaster

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of paris. Blocks of plaster, 8 in. square and 1 in. thick, were moulded with a depression in one face. The depressions were filled with larvae, soil and grass seeds; then the blocks were moistened, covered with a glass plate and kept in biscuit tins at constant temperature. A damp plaster block makes a very good container for chafer grubs, giving a saturated or near-saturated humidity, free from excessive condensed water, that remains constant over a wide range of water content. In one block (dry weight 900 gm.), first saturated with 220 gm. of water and then allowed to dry out slowly, it was found that the humidity in the depression (measured with a paper hygrometer) stayed constant at 98 per cent. until only 16 gm. of water remained in the plaster.

For Experiment 1, 56 newly hatched larvae were put in one block with a cavity measuring 6 in. \times 6 in. \times $\frac{3}{8}$ in. deep and containing a mixture of soil and grass seeds. The first examination was made at 17 days and thereafter the larvae were weighed and their food was changed at about weekly intervals until all the grubs were hibernating. At 31 days the 44 survivors were divided between two blocks and at 99 days the 17 surviving feeding grubs were reunited in one block again.

TABLE I.

Growth on germinating seeds at 15°C. (Experiment 1.)

Date	Age in days	Number of larvae surviving				Mean weight (mg.)	Range	Coefficient of variation
		First instar	Second instar	Third instar				
				Feeding	Hibernating			
7.vii.51	0	56	—	—	—	—	—	—
24.vii.51	17	52	—	—	—	19.4	14-20*	—
30.vii.51	23	4	43	—	—	22.2	16-27*	—
7.viii.51	31	—	44	—	—	49.6	35-61*	—
13.viii.51	37	—	44	—	—	69.3	40-94	17.46
20.viii.51	44	—	14	29	—	74.3	49-101	17.29
28.viii.51	52	—	1	42	—	132.4	55-202	27.56
9.ix.51	64	—	—	42	—	168.5	55-253	24.02
18.ix.51	73	—	—	34	—	176.9	112-271	21.00
21.ix.51	76	—	—	34	—	171.6	112-275	21.57
29.ix.51	84	—	—	30	—	166.4	99-251	21.81
6.x.51	91	—	—	24	3	160.0	110-246	20.42
14.x.51	99	—	—	17	7	159.7	112-224	18.62
27.x.51	112	—	—	6	6	166.6	115-230	20.58
15.xi.51	131	—	—	—	7	158.6	82-216	27.12
9.i.52	186	—	—	—	3	139.0	133-144	4.01

* At these examinations only 6 of the surviving larvae were weighed.

It seems clear from Table I that, at 15°C., the mean age at which the first moult occurs is about 20 days, with a range of 17–23 days, while the second moult takes place at about 45 days (range 40–52 days). The larvae stop feeding and hibernate at about 100 days (range 90–120 days).

The mean weight of the surviving grubs, plotted against age, gives the curve shown in fig. 1 (curve 1). The weekly gain in weight increases during the early

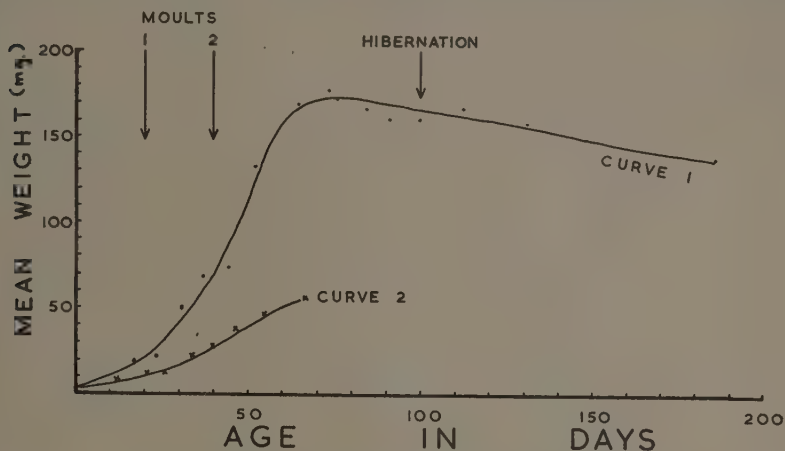


Fig. 1.—Growth curves of groups of larvae fed on germinating grass seeds at 15°C.

stages, reaches a maximum during the first part of the third instar, and tails off to nothing as hibernation approaches. Hibernation is accompanied by an immediate loss of up to 25 per cent. of total weight, due to the emptying of the gut. The use of gross weights as indicators of growth assumes that the weight of material in the gut is a more or less constant percentage of the whole, at least up to the onset of hibernation.

For Experiment 2, the depressions in the blocks were divided into compartments with interlocking aluminium strips and the growth of individual larvae, at 15°C. and fed on germinating seeds as before, was followed from hatching to the third instar (second moult). The seeds were not mixed in with the soil, as in Experiment 1, but were sprinkled on the surface of the soil just beneath the glass plate. A total of 44 first-instar larvae were cultured of which 28 survived to the first and 16 to the second moult. The moults occurred rather later than in Experiment 1, at about 26 and 60 days, respectively. The curve of mean weight for the 16 larvae that survived to the end of the experiment (fig. 1, curve 2), also shows that growth was generally slower than in Experiment 1. This was probably because the seeds were sprinkled on the surface. If the seeds are scattered throughout the soil the larvae have a constant supply of food, for they eat seeds, germinating roots or shoots indiscriminately. But with the seeds in a surface layer the larvae may be short of food until roots from the germinating seeds push down into the soil.

The successive weights of the 16 survivors are given in Table II and the individual growth curves of three of them are shown in fig. 3. The growth curve of an individual is far from being a smooth sigmoid curve like those of fig. 1. Not only does growth slow down at the moults but there may be quite large fluctuations in the rate of weight increase in the middle of an instar. Curves 1

TABLE II.
Growth of individual larvae on germinating seeds at 15°C. (Experiment 2.)

Age in days	Individual weights (mg.) of 16 larvae that survived the experiment (2nd-instar weights in italics)																Mean weight	Coefficient of variation
0	3.2	3.0	2.8	2.2	2.6	2.6	2.4	2.2	2.4								2.6	13.3
12	9.0	7.6	6.2	8.2	7.6	11.8	8.0	10.4	12.0	6.4	5.6	6.6	10.0	6.6	8.4	7.6	8.3	23.5
21	10.8	9.0	7.4	11.2	12.2	10.6	11.8	12.4	12.4	11.4	10.0	9.0	13.8	10.0	12.4	12.8	11.1	15.2
26	11.8	9.8	8.4	10.8	11.6	13.8	13.6	13.2	14.8	14.2	11.0	12.2	14.8	11.0	13.0	13.6	12.4	14.9
34	15.0	15.2	8.8	30.8	27.0	18.0	23.2	32.0	31.8	26.4	23.4	13.8	31.2	18.2	24.2	36.0	23.4	33.8
40	18	18	11	27	23	16	22	27	31	39	33	20	38	22	33	48	26.6	36.9
47	32	27	16	47	42	34	47	43	51	49	36	29	46	25	38	49	38.5	27.3
55	36	29	24	46	52	51	60	58	52	56	40	35	50	33	48	78	46.8	29.1
67	38	28	23	60	66	41	73	80	56	68	50	46	81	44	67	78	56.2	32.7

and 2 of fig. 1, therefore, do not represent the course of growth of an average larva, for the fluctuations and irregularities are smoothed out. The smooth curve nevertheless shows up one interesting point. Curve 1 of fig. 1 shows that the rate of weight increase is more or less constant during the first part of the feeding

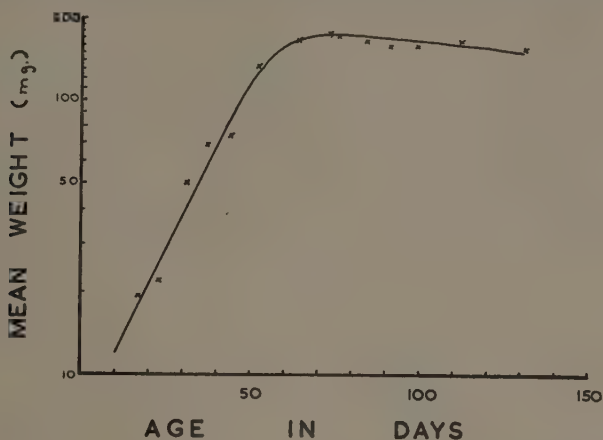


Fig. 2.—Growth curve of group of larvae (logarithmic weight scale).

third instar. If the same figures are plotted on a logarithmic weight scale (fig. 2), growth is seen to be proportional to weight during the first and most of the second instars. The two curves illustrate a point that is suggested from observation of the growing larvae. During the first two instars the grub is growing rapidly but is storing little in the fat body. All the organs and tissues of the body are growing, so that growth is proportional to weight. In the third instar, on the other hand, the grub is fully developed and is mainly occupied in storing reserves in the fat body for later use in hibernation, transformation and reproduction. The weight thus tends to increase at a constant rate.

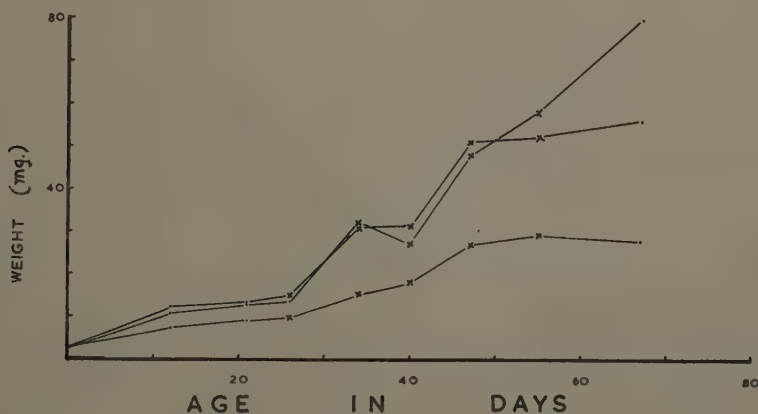


Fig. 3.—Growth curves of 3 individual larvae fed on grass seeds at 15°C. (2nd-instar weights—crosses; 1st- and 3rd-instar weights—dots).

Growth under semi-natural conditions (Experiments 3 and 4).

Growth was studied in flower-pots filled with soil from the Lake District and sown with a mixture of *Agrostis tenuis* and *Festuca ovina* (the dominant grasses of one infested field) some time before the larvae were available. Newly hatched larvae were buried in the pots and the pots embedded in the ground near Newcastle-upon-Tyne.

For Experiment 3, 48 four-inch pots were used, in each of which 6-8 larvae, all hatched the same day, were placed. At intervals throughout the late summer and autumn, one or more pots were taken at random and the surviving grubs extracted and weighed. Thus the figures shown in Table III (and in fig. 4 by dots) are for different groups of larvae of various ages, each group having been left undisturbed from the time of hatching.

For Experiment 4, 24 seven-inch pots were bedded out. On July 3rd, 96 weighed first-instar larvae, hatched between June 29th and July 3rd, were put into six of the pots, 16 grubs per pot. On 1st August, the surviving larvae were

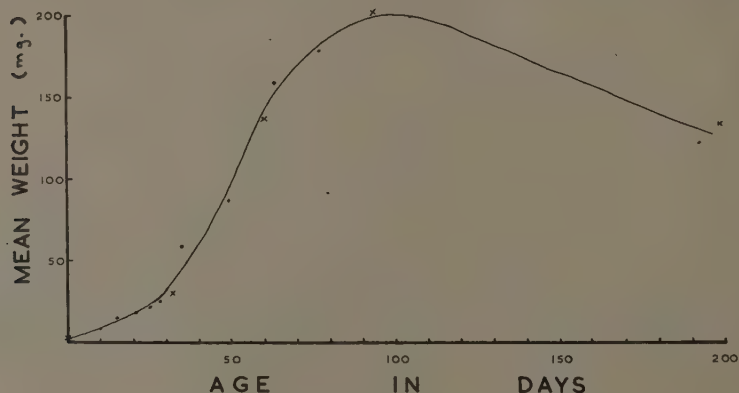


Fig. 4.—Growth curve for groups of larvae reared under semi-natural conditions. (Data of Experiment 3—dots; data of Experiment 4—crosses.)

extracted, weighed and returned to six of the unused pots. This procedure was repeated on 29th August and 1st October. On 14th January the larvae were extracted and weighed for the last time. The experiment thus gives the successive weights of the same five groups of larvae at five points in the growth period.

Mortality was fairly high in Experiment 4. Only 37 of the original 96 survived until 14th January. Mortality per pot over the whole period of the experiment varied considerably but no more than in other experiments carried out under similar conditions. Again, looking at the whole group of 96 larvae, the number of deaths between each examination varied little throughout the experiment. There did not seem to be any tendency for mortality to be higher among young than among old larvae or *vice versa*. The different pots showed considerable variation, however. In the first two months there were so many deaths in pots 2 and 4 that at the second examination the grubs were put together in one pot (and for this reason are shown as "Pots 2, 4" all through Table IV). The other four pots lost less than 25 per cent. of their grubs in the first two months. Between the second and fourth examinations, on the other hand (*i.e.*, between 29th August and 14th January), while pots "2, 4", 3, 5 and 6 showed little mortality, pot 1 lost all but one of its grubs.

TABLE III.
Growth of larvae under semi-natural conditions in four-inch flower-pots. (Experiment 3.)

Number of pots	Larvae put in	Number of larvae surviving					Survival (%)	Age in days	Mean weight (mg.)	Standard error
		First instar	Second instar	Third instar						
				Feeding	Hibernating					
1	6	6	—	—	—	100	10	8.7	0.74	
1	5	5	—	—	—	100	15	14.6	1.36	
1	6	6	—	—	—	100	21	17.6	0.60	
4	24	3	20	—	—	96	25	20.9	0.97	
2	12	1	11	—	—	100	28	25.2	2.72	
1	6	—	5	—	—	83	35	59.4	5.44	
1	6	—	1	5	—	100	49	86.3	5.88	
1	7	—	—	6	—	86	63	159.2	20.54	
1	6	—	—	5	—	83	78	176.8	13.46	
3	19	—	—	7	2	47	104	198.6	10.63	
16	101	—	—	—	21	21	192	120.6	10.40	

In Experiment 3, mortality was definitely less among young larvae. Until about 60 days (early in the third instar) it was usual to recover as many grubs as had been put into the pot as first-instar larvae. After 78 days the numbers recovered became very low (see Table III). It was also noticed that at about 60 days the grass in these pots became more or less completely undercut by the larvae. It is likely that the undercutting which leads to the formation of a layer of loose crumbly soil just below the bases of the grass stems is responsible for the increased mortality, not so much through starvation as by allowing the larvae greater freedom of movement which leads to an increase in cannibalism. This point is discussed further on p. 151. The results for Experiment 4 are shown in Table IV and the mean weights for the whole group on the five occasions are included in fig. 4 (crosses).

In Experiment 4, all the larvae were in the second instar at the second weighing (32 days after hatching). At the third weighing (60 days) all larvae except two had moulted to the third instar. At 93 days not one of the grubs was hibernating. Experiments 3 and 4 thus confirm that the moults occur at about 3-4 and 7-9 weeks, respectively, and that the grubs hibernate at 100-120 days after hatching.

Growth on different plants (Experiment 5).

Previous authors, writing of the Garden Chafer in England, have assumed that it feeds on grass roots. Rittershaus (1927) states that in Germany, though she usually found the grubs on grass roots, she had also found them feeding on the roots of a wide variety of other plants such as cereals, brassicae, alpine plants, trees and shrubs. Other scarabs are known to feed fairly indiscriminately on roots of all kinds, humus, dung, etc., and it would be surprising if *P. horticola* were confined to one or even a group of plant species. The following experiment was designed to show whether the larvae could survive on a number of different pasture plants.

Fifty seven-inch pots were sown or planted (depending on the availability of seeds and plants) with pure stands of 13 species of herb to be found in typical chafer-infested pasture. Two grasses (*Agrostis tenuis* and *Festuca ovina*) were included as controls. Three weeks to a month later, six newly hatched larvae were buried in each pot. The pots were bedded out as in Experiments 3 and 4 and left until the end of November, when the surviving hibernating grubs were extracted, counted and weighed. The pots were "weeded" frequently to keep the plant cultures pure. The data obtained are shown in Table V. Of the 300 grubs cultured, 102 (33%) survived to the hibernating stage. This overall figure compares favourably with survival in Experiments 3 and 4.

The survival of one grub of a small group feeding on a particular plant can be taken to mean that survival is possible on the roots of that plant. The Lake District soil used in the experiment is not rich in humus, and larvae are unable to survive in it for long unless growing roots are available. The only other source of food would be the occasional seedlings (of grasses and other weeds), self-sown in the pots between the "weeding" inspections. It seems unlikely that a grub could grow to maturity on so sparse a diet.

On the other hand, the death of all the grubs in a pot does not necessarily imply that the roots are unsuitable for growth; in the eight control pots the number of grubs surviving in a pot varied from 0 to 4. There may be causes of mortality that are indirectly due to the plants though not connected with the suitability of the species for growth. For example, spindly plants may leave a large area of the soil exposed and so cause abnormally wide fluctuations in the moisture and temperature of the surface soil. Different types of root systems may also affect soil structure.

Only two species of plants failed to support any larvae at all. These were hop trefoil (4 pots) and wood sorrel (2 pots). In the former case, the plants grew

TABLE IV.
Growth of larvae under semi-natural conditions in seven-inch flower-pots. (Experiment 4.)

Date	Age in days	Pot 1			Pots 2, 4			Pot 3			Pot 5			Pot 6			Total		
		N	Mean weight (mg.)	C	N	Mean weight (mg.)	C	N	Mean weight (mg.)	C	N	Mean weight (mg.)	C	N	Mean weight (mg.)	C	N	Mean weight (mg.)	C
3.vii.51	0	16	3.2	10.51	32	2.8	13.22	16	2.7	15.49	16	3.0	11.50	16	2.9	12.09	96	2.9	12.71
1.viii.51	32	15	42.8	35.75	13	20.9	30.70	15	28.4	31.39	14	29.0	26.64	12	25.1	32.45	69	29.6	33.84
29.viii.51	60	15	169.7	21.44	9	95.9	37.30	13	127.4	28.24	*9	141.3	23.98	12	115.3	22.78	58	133.1	23.98
1.x.51	93	12	214.0	10.45	8	195.1	18.42	12	201.3	17.24	10	208.9	11.95	11	184.0	15.71	53	201.1	14.67
14.i.52	198	1	146.0		6	135.5	11.11	12	137.9	17.49	10	136.3	12.38	8	119.0	6.45	37	133.2	13.60

N = number of larvae contributing to the mean weight. C = coefficient of variation.
* On this occasion 11 larvae were found in pot 5, of which only 9 were weighed.

TABLE V.
Growth and survival of larvae fed on different species of plants. (Experiment 5.)

Plant species	Number of pots	Number of 1st-instar larvae inserted (11.vii.53)	Number of 3rd-instar larvae surviving (24.xi.53)	Mean weight of survivors (mg.)	Standard error
Yarrow (<i>Achillea millefolium</i>)	6	36	24	147.5	5.72
Florn grass (<i>Agrostis tenuis</i>)	4	24	12	144.1	12.57
Sheep's fescue (<i>Festuca ovina</i>)	4	24	12	175.4	10.52
White clover (<i>Trifolium repens</i>)	4	24	9	173.2	12.73
Ribwort (<i>Plantago lanceolata</i>)	5	36	13	151.1	12.43
Mouse-ear chickweed (<i>Cerastium vulgatum</i>)	3	18	1	166.0	—
Hop trefoil (<i>Trifolium campestre</i>)	4	24	0	—	—
Germander speedwell (<i>Veronica chamaedrys</i>)	4	24	8	174.5	17.99
Field woodrush (<i>Luzula campestris</i>)	2	12	3	212.5	32.94
Crowfoot (<i>Ranunculus acris</i>)	2	12	2	226.5	20.51
Selfheal (<i>Prunella vulgaris</i>)	2	12	9	180.4	9.19
Wood sorrel (<i>Oxalis acetosella</i>)	2	12	0	—	—
Birdsfoot trefoil (<i>Lotus corniculatus</i>)	2	12	1	181.0	—
Heath bedstraw (<i>Galium saxatile</i>)	2	12	2	165.5	12.50
Ladies' bedstraw (<i>Galium verum</i>)	2	12	3	136.7	8.69
Tornientil (<i>Potentilla erecta</i>)	1	6	1	191.0	—
Sorrel (<i>Rumex acetosa</i>)	1	6	2	140.5	7.75

well for the first few weeks of the experiment but gradually declined in vigour and finally died out almost entirely. Clearly the larvae died from a lack of food rather than from its unsuitability. In the case of wood sorrel there were plenty of roots to be found when the pots were examined in November, the soil was moist and had never seemed too dry at any of the "weeding" inspections, and it is thus possible that larvae are unable to survive on the roots of this species of plant. However, there were only two pots (12 larvae in all) in this group, and since 100 per cent. mortality occurred in some control pots for no apparent reason, the death of all the larvae in the wood sorrel pots may also have been due to chance. Alternatively, as this plant shows very little top growth, this may have lead to an unsuitable soil environment under a pure stand of the plant.

The hibernating larvae were weighed and their weights are included in Table V. Different species of plants do not appear to affect to any appreciable extent the final weight (growth) of the larva that has fed upon them. An analysis of variance carried out on the results from the 23 pots representing the first five species of plants in Table V gave a value for the variance ratio (variation between plant species/variation within pots) of $F = 2.22$ (P approx. 0.08). In other words, these plant species did not differ significantly in their suitability for larval growth.

The Weight of the Hibernating Larva and Pupa.

The previous section gives a general picture of the growth of larvae through the late summer and autumn. In November and December, the fully fed larvae empty the gut and burrow down to spend the winter in a quiescent state. Field samples of these hibernating larvae and (in the spring) of pupae show considerable variation in mean weight, representing a large variation in reproductive capacity.

The regression of fecundity (F) on pupal weight (W) in a random sample of 160 females was $F = 0.113W - 6.60$ (Milne & Laughlin, 1956). Thus for every 9 mg. increase in pupal weight an extra egg is laid. The total range in weight of female pupae in Table IX below is 72–310 mg., equivalent to a range of 27 eggs. With an average fecundity of about 13 eggs per female (Milne & Laughlin, 1956, p. 13), this represents a substantial variation in reproductive capacity.

The weight of the hibernating larva.

A small field sample of hibernating larvae (with gut already empty) was collected on 18th December 1948. The larvae were weighed, kept in soil-filled glass tubes embedded in the earth in the open until all had pupated (16th May 1949), and weighed again. Fourteen males and eight females survived, and the mean loss in weight over the five months was 27.1 mg. (range 15–44 mg.) for males, *i.e.*, from 159.1 mg. in December to 131.9 mg. in May, and 38.8 mg. (range 31–51 mg.) for females, *i.e.*, 186.1–147.4 mg. These losses represent 17.1 and 20.8 per cent. (males and females, respectively) of the weight at the beginning of the winter.

From October to November 1953 a series of samples was taken for an experiment to be described below (p. 145, (c)). The first sample was taken on 16th October when only 15 per cent. of all the larvae dug up were hibernating. The hibernating larvae were weighed and kept in plaster blocks over the winter, being reweighed at about monthly intervals until they pupated in April. Sixty larvae were kept, of which 51 (47 males and 4 females) survived to the pupal stage. The last sample was taken on 17th November when 79 per cent. were hibernating. On this occasion only feeding grubs and those in process of emptying the gut were kept. Of 52 such larvae, 22 (12 males and 10 females) survived to the pupal stage. A summary of the data from these two samples is given in Table VI and is shown graphically in fig. 5. Up to the turn of the year, weight was lost fairly rapidly. In the three months that preceded pupation the mean weight of the

TABLE VI.
The weight lost by hibernating larvae during the winter.

Date of collection	Number of larvae surviving the winter	Mean weight in mg. (and standard error) at monthly intervals through the winter						Total loss in weight (%)	
		21.x.53	17.xi.53	30.xi.53	14.xii.53	11.i.54	8.ii.54		April 1954 (pupae)
16.x.53	47 ♂♂	203.5 (3.37)	185.3 (3.11)	170.1 (2.83)	166.6 (2.68)	159.1 (2.57)	161.1 (2.61)	152.5 (2.71)	25.00
	4 ♀♀	256.3 (29.04)	244.0 (26.84)	220.8 (21.85)	215.8 (22.27)	208.8 (21.29)	211.0 (21.26)	200.8 (21.20)	21.66
17.xi.53	12 ♂♂	—	—	168.8 (6.50)	153.0 (5.79)	138.8 (4.46)	143.4 (4.45)	132.4 (4.94)	21.61
	10 ♀♀	—	—	204.2 (10.72)	183.0 (9.64)	169.9 (8.30)	171.8 (8.49)	160.5 (9.46)	21.43

larvae remained fairly constant though the behaviour of individuals varied widely, some grubs even gaining in weight, in an extreme case to the extent of 11 mg. in 28 days.

From the foregoing, it is obviously necessary, in comparing weights of hibernating larvae, to take into account the date of collection. Samples taken before Christmas are of little value except for comparison with other samples taken from the same population at the same time. Even in the three months that precede pupation, caution is needed when comparing the weights of samples.

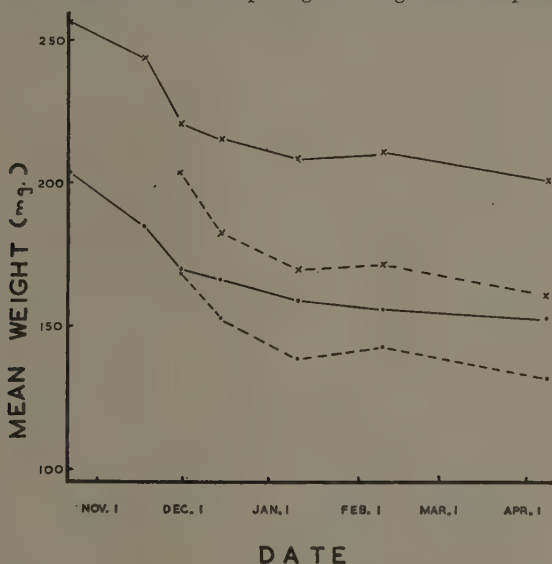


Fig. 5.—Overwinter loss of weight in hibernating larvae. (Males—dots. Females—crosses. Unbroken line—1st sample, 16th October. Broken line—4th sample, 17th November.)

The weight of the pupa.

Individual pupae are practically constant in weight up to the time of emergence. Thus five male and four female pupae, weighed on the day they pupated, on four occasions during the pupal period and again just before the adults emerged, showed a barely perceptible loss in weight, the mean per pupa being 2.9 mg., range 1–5 mg. This is a good reason for using pupae for the comparison of field sample weights; another is that pupae can be sexed easily. The sex ratio of field populations (see Milne, 1956) varies quite widely and that of samples very widely; females are, on the average, heavier than males. The difference between male and female mean weights in the samples recorded in Tables IX and X varies from 8.4 to 66.6 mg. Two samples differing in sex ratio could differ significantly in their mean weights when a comparison of the corresponding sex would show no difference.

Several of the collections listed in Table IX and X were taken in winter and kept until the larvae pupated. There were wide variations in the temperature treatments for different collections, both deliberate and fortuitous, and it might be thought that such changes could affect the weight of the pupa. Evidence on this point is provided by an experiment in which 1,000 larvae were collected in December 1952, weighed and kept at 0–10°C. until the middle of January. Thereafter, while the main stock remained at the low temperature, groups of 48 were

TABLE VII.

The effect of the date of pupation on the mean pupal weight (in mg.).

Group number	1	4	5	6	7	8	9	10	11	12	13	14	15	16
	Mean date of pupation (approximate)	11.ii	12.ii	14.ii	15.ii	21.ii	25.ii	1.iii	7.iii	14.iii	20.iii	27.iii	2.iv	11.iv
Males	Number	10	20	18	17	30	25	26	24	27	16	22	22	18
	Mean weight	136.4	144.3	143.7	134.4	145.8	142.4	137.7	143.8	143.6	138.8	143.1	144.2	136.6
Females	Number	23	13	22	18	13	17	20	19	19	21	23	23	22
	Mean weight	173.0	179.5	173.2	173.1	176.9	182.9	171.4	164.8	166.4	176.1	164.1	168.5	171.3

taken at random each week and incubated at 15°C. Under this treatment the groups pupated, one after another, over a period of about three months, and one might expect that the later groups would have been considerably lighter. The figures from this and other similar cultures, however, indicate that this was not the case. The mean pupal weights for the 14 groups are remarkably constant and show no tendency to decrease when pupation is delayed (Table VII).

The use of pupal weights after keeping the larvae over the winter raises a further question. If mortality in culture is correlated with weight, an error will be introduced, greater or less according to the percentage of deaths over the winter. However, some larvae were weighed at the time of collection and we can compare the initial larval weights of those that died and those that survived. Table VIII indicates that mortality is not selective.

TABLE VIII.

The weight when collected of larvae that subsequently die or that pupate.

Date of collection and weighing	Non-survivors			Survivors		
	Number	Mean weight (mg.)	Standard error	Number	Mean weight (mg.)	Standard error
10.xii.49	50	198.4	5.51	107	203.1	4.07
8.xii.49	17	167.1	6.38	68	166.2	2.79
14.xii.52	127	167.7	3.09	793	171.1	0.99
16.x.53	9	222.1	18.79	51	207.6	4.19

It seems, therefore, that the weight of the pupa is the most useful index available of the size of the individual chafer. It is a measure of the growth achieved the previous summer and autumn and constitutes a datum line between the larval growth period and the adult reproductive stage. In the discussion below it will be used in preference to the weight of the hibernating larva except in a few cases where samples taken from the same population at the same time are to be compared.

Variation in pupal weight.

During 1950-1953, collections were made in two fields in the Lake District, one at Ambleside and one at Buttermere. Neither field was uniform in vegetation, soil moisture or topography. However, at Ambleside all the collections were made in a flat and fairly uniform part of the field while at Buttermere three apparently homogeneous areas (called Buttermere 1, 2 and 3 in Tables IX to XI) were sampled. The first object of the collections was to provide stocks of larvae, pupae or adults for experiment. To give information about population density (which will be the subject of a later paper by Dr. A. Milne) the larvae or pupae were extracted from separate square yards of turf sited at random in the collection area. With the method of collection so fixed the weights are essentially a by-product and only a limited amount of information can be gained from them. Samples were taken during the winter and the hibernating larvae allowed to pupate before weighing.

The data are summarised in Table IX. The mean and (in brackets) range of the pupal weights recorded are 139.3 mg. (65-242) for males and 171.3 mg.

(72-310) for females. The variation in weight from year to year and from place to place is obviously considerable. Thus, the pupae collected in 1950 from Buttermere 3 were very heavy (means: male, 167 mg., female, 234 mg.). Those collected in the same area three years later were about normal weight (means: male, 135 mg., female, 174 mg.).

TABLE IX.

The pupal weights of field-collected individuals.

Collection area	Year of pupation	Male			Female		
		No. of pupae	Mean wt. (mg.)	Range (mg.)	No. of pupae	Mean wt. (mg.)	Range (mg.)
Buttermere 1 ..	1951	173	110.0	75-145	143	131.1	72-196
Buttermere 1 ..	1952	164	149.1	92-193	123	188.5	135-235
Buttermere 1 ..	1953	172	142.2	99-183	131	173.8	133-214
Buttermere 2 ..	1950	503	139.4	95-180	418	176.1	112-265
Buttermere 3 ..	1950	38	167.1	135-202	43	233.7	157-310
Buttermere 3 ..	1953	17	134.7	108-148	14	173.6	154-202
Ambleside ..	1952	331	142.4	91-225	214	175.8	125-243
Ambleside .. (2 collections)	1953	794	140.3	65-242	690	167.9	85-245
Total		2192	139.3	65-242	1776	171.3	72-310

The analysis of this variation presents some difficulty since the weights of pupae from the same square yard are correlated one with another. This correlation is to be expected, for it is a general rule, exemplified notably in the yields of crop plants, that adjacent individuals vary less than those widely separated. Whether this rule applies to the present data can be tested by comparing the variance of the mean weights of the square yards with the variance of pupal weights within the square yards. In Table X is shown such of the data from Table IX as could be allocated to particular square yards. Calculations of the variances show that in most area-years the variance between square yards is high compared with that within them. Thus, the Ambleside (March 1953) collection gave a variance ratio, F , of 13.19 for males and 19.46 for females, with $P = 0.001$ in both cases. Evidently the weights of pupae from the same square yards are correlated, and cannot validly be treated as independent random samples of the whole collection area.

Comparisons of the pupal weights representing different area-years must be based upon the square-yard sampling units themselves, which are independent and random, and of which the best estimates are the square-yard means. Such data are limited in number owing to the fact that for many of the pupae a record was made only of their weight and not of from what square yard they came. The data available are set out in Table X, and a summary is given in Table XI of the mean values of the pupal weight for the different collection areas and years, being, for each sex, the mean of the square-yard means given in the preceding Table.

TABLE X.

The mean weight of pupae from square-yard sampling units.

Collection area	Year of pupation	Male			Female			*Sex ratio
		No. of pupae	Mean wt. (mg.)	Range	No. of pupae	Mean wt. (mg.)	Range	
Buttermere 1 ..	1951	26	119.3	75 - 140	23	136.3	95 - 173	46.9
		29	104.3	83 - 127	15	118.9	101 - 146	34.1
		28	115.7	95 - 145	26	134.8	72 - 179	48.2
		24	102.3	84 - 124	20	127.2	74 - 149	45.5
		37	106.7	78 - 135	29	129.9	86 - 196	43.9
		11	108.1	88 - 141	20	130.5	91 - 179	64.5
		18	115.1	97 - 141	10	140.2	119 - 176	35.7
Buttermere 1 ..	1953	18	141.8	122 - 158	12	164.0	143 - 183	47.6
		11	139.8	126 - 154	11	166.8	133 - 197	44.8
		0	—	—	3	162.7	142 - 179	50.0
		13	129.9	99 - 150	5	154.2	135 - 199	40.6
		47	142.9	124 - 183	39	174.5	137 - 214	44.8
		6	146.5	136 - 157	5	180.2	171 - 196	50.0
		0	—	—	2	187.0	182 & 192	57.1
		2	144.5	133 & 156	2	174.0	160 & 188	28.6
		9	138.6	116 - 162	4	192.5	158 - 205	26.3
		8	140.9	124 - 150	11	178.0	159 - 201	56.3
		11	150.6	128 - 166	5	179.0	168 - 193	29.2
		11	143.5	133 - 155	14	175.4	155 - 197	58.3
		15	141.7	114 - 165	5	165.6	156 - 186	29.0
		21	145.6	118 - 181	13	182.0	149 - 203	34.9
Buttermere 2 ..	1950	18	153.3	122 - 180	21	189.6	148 - 235	54.5
		14	128.6	116 - 148	8	168.8	147 - 205	36.4
Buttermere 3 ..	1953	17	134.7	108 - 148	11	172.3	154 - 202	43.1
		0	—	—	1	177.0	—	40.0
		0	—	—	2	179.0	177 & 181	100.0
Ambleside (collected December 1952)	1953	19	131.9	83 - 154	16	164.4	132 - 192	45.7
		63	142.2	116 - 177	48	176.8	85 - 213	43.2
		93	137.5	89 - 179	75	167.2	120 - 211	44.6
		21	133.0	95 - 162	28	155.7	118 - 184	57.1
		37	151.5	118 - 242	37	192.2	125 - 235	50.0
		20	154.8	133 - 180	12	195.1	171 - 225	37.5
		49	140.5	109 - 173	53	167.2	102 - 213	52.0
Ambleside (collected March 1953)	1953	31	149.3	109 - 183	23	192.0	149 - 240	42.6
		24	124.9	65 - 152	27	135.2	103 - 177	52.9
		48	126.2	93 - 157	25	151.4	111 - 176	34.3
		18	125.4	90 - 153	18	133.8	117 - 171	50.0
		12	134.6	110 - 159	11	143.0	112 - 165	47.8
		15	121.0	90 - 135	22	142.1	104 - 182	59.5
		66	149.1	108 - 202	48	177.6	128 - 220	42.1
		64	151.0	117 - 188	60	180.8	117 - 241	48.4
		36	136.9	96 - 180	40	151.1	112 - 217	52.6
		49	136.8	95 - 160	50	165.4	107 - 214	50.5
		71	144.9	106 - 182	71	178.8	125 - 245	50.0
		13	135.3	109 - 178	12	154.1	130 - 181	48.0
		25	128.3	101 - 194	5	158.8	146 - 176	16.7
		20	150.9	103 - 174	9	171.3	141 - 197	31.0

* The percentage of females in the total number of pupae in the sample (which is not necessarily the same as the total number weighed).

An analysis of variance of the data in Tables X and XI shows that the area-years differ significantly, the value for the variance ratio (variance between area-year means/variance between square-yard means within area-years) being $F = 17.39$ ($P = 0.001$) for males, $F = 14.81$ ($P = 0.001$) for females. The results of t -tests on pairs of means are also indicated in Table XI. It will be noted that Buttermere 1 yielded heavier pupae of both sexes in 1953 than in 1951.

TABLE XI.

Pupal weights in different areas and years (means of the square-yard mean weights given in Table X).

Collection area	Year of pupation	Males			Females		
		N	Mean weight (mg.)	S.E.	N	Mean weight (mg.)	S.E.
Buttermere 1 ..	1951	7	110.2*	2.46	7	131.1*	2.60
Buttermere 1 ..	1953	12	142.2	1.49	14	174.0	3.93
Buttermere 2 ..	1950	2	141.0	12.00	2	179.2	10.49
Buttermere 3 ..	1953	1	134.7	—	3	176.1	2.08
Ambleside (March 1953 collection)	1953	14	136.7	2.76	14	159.7	5.11

N = Number of square-yard sampling units. S.E. = Standard error of mean.

* Significantly lower than the other values for the same sex, which do not differ significantly among themselves.

Factors affecting variation in pupal weight.

In nature, the weight of the pupa varies widely from year to year, from place to place and within each apparently homogeneous area. This variation plays an important part in the ecology of the chafer, for the fecundity of the adult female depends very much on the pupal weight, that is, on the reserves laid down by the feeding larva. The causes of this variation are largely unknown at present but the work described below suggests some of the factors responsible.

The effect of hatching and hibernation date.

(a) The pupae from Buttermere 1 in 1951 and 1953 were the products of the larval growth periods of 1950 and 1952, respectively. Setting down the available data for these growth periods (Table XII), it is at once obvious that there were no great differences in temperature or rainfall between the two years, except that September 1950 was a very wet month and November 1952 rather dry. The only large difference between the two growth periods was that in 1952 (the year that produced the heavier pupae) the start of the adult flight season (hence the laying and hatching of eggs) and the start of hibernation of fully fed larvae were both some three weeks earlier than in 1950. Thus, while the growth period was of about the same duration in the two years, in 1952 it was put forward.

(b) An experiment which duplicated this displacement in time showed a similar variation in weight. Larvae (254) were cultured outdoors in pots sown or planted with grass (as in Experiment 4, on p. 132). The eggs were laid by a batch of females which had been divided into two lots at random, one being

allowed to emerge normally, the other being cooled as pupae to retard development. Half the larvae hatched around 1st July 1951 and the other half about 23rd July, about three weeks later. The pots were left undisturbed until, on 7th November, some were examined and it was found that, while about 80 per cent. of the early-hatched larvae were hibernating, only 1 out of 14 grubs taken from the "late" pots looked like a hibernating larva. Nineteen days later, on

TABLE XII.

Climate and timing of growth period at Buttermere 1.

					1950	1952
*Mean monthly ground temperature (°F.)						
July	60.3	60.4
August	59.5	58.9
September	54.2	51.9
October	49.3	47.4
November	41.5	39.8
Total monthly rainfall in inches and (in brackets) percentage of average						
July	3.41 (89)	3.99 (104)
August	8.06 (154)	8.55 (164)
September	14.51 (343)	6.01 (142)
October	5.66 (101)	5.67 (101)
November	6.77 (120)	3.21 (57)
Date on which adult flight season started					2nd June	22nd May
Date on which the larval population started to go into hibernation					30th October	9th October
Mean weight (mg.) of pupae the following spring (i.e., 1951 and 1953, figures from Table XI)					Male	110.2
					Female	131.1
						142.2
						174.0

* The average of mean air temperature and mean temperature at 1-foot depth in the soil.

26th November, the rest of the pots were lifted. Of the "early" larvae, 92 per cent. were hibernating, but of the "late", only 56 per cent. Thus the three weeks' delay in hatching was apparent and perhaps somewhat extended in the hibernation dates of the two sets of grubs.

The weights of hibernating "early" larvae lifted on 7th November were compared with the weights of hibernating "late" larvae lifted on 26th November (Table XIII). The means were 154.2 and 120.6 mg., respectively, and the difference is significant ($P = 0.001$). As noted on p. 139 it is not always wise to compare the weights of larvae at the beginning of the hibernation period since the grub loses weight rapidly in the first few weeks. There is little danger in the comparison here since the "early" larvae were further advanced on 7th November (80 per cent. hibernating) than were the "late" larvae on 26th November (56 per cent. hibernating). Thus, if there were no real difference between the two groups (i.e., no difference in total growth) the "early" group would, if anything, be the lighter. Furthermore, some of the grubs from this experiment were not weighed until the middle of January (see Table XIII), when the "early" larvae were again significantly the heavier ($P = 0.001$).

(c) In nature, a population of third-instar larvae takes five or six weeks to enter hibernation. The first larvae start hibernation about the middle of October, the bulk of the population in October–November and a few laggards may

still be found feeding in December if the winter is mild. From (a) and (b) above, it seems likely that the first grubs to hibernate will be the earliest hatched, even within one population. A series of samples was taken in the winter of 1953-54 from a section of the Ambleside collection area. The section was sampled at about weekly intervals throughout the "change-over period", i.e., the time

TABLE XIII.

The effect of time displacement of the growth period on the weight of hibernating larvae.

Date of hatching		"Early" larvae	"Late" larvae
		1st July	23rd July
Percentage of hibernating larvae on date given	7th Nov.	81	7
	26th Nov.	92	56
Number (N) and mean weight in mg. (\bar{x}) of hibernating larvae on date given	7th Nov.	N \bar{x} Range	
		35 154.2 101 - 204	
	26th Nov.	N \bar{x} Range	25 120.6 85 - 168
	11th Jan.	N \bar{x} Range	12 97.8 72 - 139
		15 125.7 96 - 155	

during which the population was going into hibernation. The hibernating grubs in each sample were weighed, kept over the winter, and sexed and re-weighed when they pupated.

On 6th October 1953 the field had been sampled for another purpose and of the total of 193 grubs found only 2 were hibernating. Ten days later, on 16th October, the first of the series of samples was taken. The method of sampling

TABLE XIV.

The sex ratio and weights of pupae formed in 1954 from larvae taken during the hibernation period, 1953.

Collection date			17.x	23.x	3.xi	17.xi
Hibernating larvae/total collected %			15.5	38.8	42.5	78.8
No. of pupae ♂			47	41	70	12
♀			5	16	25	12
Sex ratio (♀ per cent.)			9.6	28.1	26.3	50.0
Numbers (N) and mean weights (x) of pupae	♂	N	47	38	66	12
		\bar{x}	152.5	146.0	148.0	132.4
		S.E.	2.71	2.44	1.88	4.94
	♀	N	4	13	25	10
		\bar{x}	200.8	183.4	190.7	160.5
		S.E.	21.20	3.77	4.06	9.46

S.E. = Standard error of mean.

was to take each one of the series from the same section of the field, an area about 100 ft. in diameter where the population density was fairly high. On each occasion 10–15 separate sq. ft. of the sward, sited at random within the area, were dug up. The numbers of grubs feeding, in process of emptying the gut and in hibernation were counted. On the first three occasions the two latter classes were kept. On the last occasion, 17th November, only feeding grubs and grubs emptying the gut were kept.

The results of these observations are shown in Table XIV. It will be noticed that the numbers of male and female pupae in each sample do not always tally with the number of pupal weights given. This is because some of the larvae usually fail to pupate successfully in the plaster blocks (a rare occurrence in nature), or die when still in the prepupal stage. These individuals cannot be weighed but they can usually be sexed, hence the discrepancies in the Table. There is a definite tendency for males to predominate in the earlier samples. This has important ecological implications which are discussed below (p. 150). At the moment we are concerned with the pupal weights of larvae from the four samples. An analysis of the weights gave values for the variance ratio (variation between samples/variation within samples) of $F = 4.98$ (males) and 4.86 (females), $P = 0.01$ in both cases. Results of t -tests at the 5 per cent. level of probability showed that pupae from the first three samples were significantly heavier than those of the corresponding sex from the last sample, although the three samples did not differ amongst themselves. It must be remembered that while the first sample contained only the earliest hibernating larvae and the last sample was restricted to the laggards, the middle two samples included all the larvae that had hibernated before the date of their collection. It is to be expected, therefore, that the weights of pupae from the middle two samples should not differ significantly from the first.

From the foregoing observations, it seems fairly certain that time-displacement of the growth period is a major cause of variation in pupal weight. The details of the mechanism can only be guessed at present. It is obvious, for example, that the early larvae will be subject to higher temperatures than late larvae, since the temperature is falling throughout the late summer and autumn; perhaps the grubs feed more actively at higher temperature. Also, the quality and quantity of the roots of pasture plants change with the seasons and may affect the growth of the grubs.

The effect of different plants.

Experiment 5 (p. 134) gave no reason to suppose that roots of different plants affect the growth of larvae. However, another experiment did give a positive result.

Early third-instar larvae (144), collected from the field on 31st August, were weighed and buried, six to a box, in 24 wooden boxes (12" × 15"), of which 8 had previously been sown with ryegrass, 8 with wild white clover and 8 with lettuce. On 10th December, well after the end of the growth period, the hibernating grubs were re-weighed, and kept over the winter until they pupated. The pupae were then weighed and sexed. Thus there are four sets of weights: those of the feeding larvae before the experiment, the surviving hibernating larvae and the male and the female pupae. The four sets are summarised in Table XV.

There is obviously no difference between the mean weights of the three groups of larvae when collected from the field. The mean weights of the groups of hibernating larvae show a much greater variation, however, those fed on lettuce seedlings being significantly heavier than those on clover or ryegrass ($P = 0.01$), though the difference between the two latter groups is not significant.

The above is true for both male and female pupae and suggests that different foods can affect the weight of the pupa. However, as was emphasised on p. 134

TABLE XV.
Larval and pupal weights (mg.) of individuals fed on different plants.

	Clover			Lettuce			Ryegrass		
	N	\bar{x}	Range	N	\bar{x}	Range	N	\bar{x}	Range
Early 3rd-instar larvae before the experiment	48	153.0	81 - 195	48	152.4	62 - 197	48	151.9	83 - 193
Hibernating larvae weighed on 12th December	39	150.6	122 - 191	39	194.7	156 - 250	38	153.6	120 - 209
Male pupae	18	125.8	105 - 148	18	143.9	126 - 163	17	132.1	109 - 167
Female pupae	7	139.7	124 - 171	15	193.4	158 - 220	6	161.3	147 - 194

N = Number of individuals. \bar{x} = Mean wt.

(Experiment 5), to present larvae with pure stands of different plants varies not only the food they eat but also, perhaps, the conditions under which they live. This experiment should be repeated under more strictly controlled conditions before the observed variation in weight is accepted as a direct result of variation in food.

The effect of population density.

In nature, a heavy population of grubs will undercut the vegetation more or less completely. Birds, searching the loosened turf for grubs, or grazing animals tugging at the grass, may then expose quite large areas of bare soil. Several sets of field samples were taken and a number of experiments carried out in an attempt to discover if high density and/or its sometimes attendant barring of the soil affected the growth of the larva and hence the weight of the hibernating grub and pupa.

Raw (1951, p. 612) investigated the point by collecting individuals from undamaged and severely damaged parts of the same field and comparing their weights. He does not give the population densities involved nor does he say whether by "severely damaged" he means bared soil or undercut vegetation. The data recorded below indicate that this point might be of importance. Raw found a significant difference between the weights of individuals from undamaged and "severely damaged" areas.

On the 23rd September 1949, population density in the fields under observation at Buttermere was high. There was much loose turf and a good deal of bird damage. Samples of grubs were taken from bare soil, from undercut but undisturbed turf and from firm turf. The larvae were weighed and their weights compared. Three sets of samples were taken:

Set 1. Seven adjacent sq.-ft. samples forming an L-shape on the ground. Sample F, at the angle of the L, covered a bare patch of soil. Samples G, H, I and K, L, M, respectively, formed the two arms of the L and ranged from loose, undisturbed turf to firm grass, apparently unaffected by the larvae beneath.

Set 2. Five adjacent sq.-ft. samples in a straight line. Samples A and B were on bare soil and samples C, D and E on fairly firm, undisturbed sward.

Set 3. A small bare patch, roughly circular and about 12 in. in diameter was chosen. Taking the middle of the patch as centre, a circle of 6-in. radius was excavated (sample N) and the grubs extracted. Three more concentric circles of radii 1 ft., 18 in. and 2 ft. (samples O, P and Q, respectively) were then taken up.

The numbers and weights of the larvae found in the three sets of samples are shown in Table XVI. There is little difference between the weights of larvae from bare patches and from the surrounding turf, undercut or firm. Nor is there any relation between the number of larvae found (sample density) and the mean weight of the sample.

One point does emerge from these figures. The sample density, expressed as grubs per square foot of sward, tends to be low in bare patches, high in the surrounding loose turf and to decrease the further the samples are from a bare patch. One would expect a low density in the bare patches where the birds have been at work but it may also be that the grubs move out from a centre of high population, especially when the top cover has been removed.

Since these figures were inconclusive, another and more extensive set of data was obtained on 16th November the same year. Larvae (508) were collected from two trenches 35 ft. by 6 in. by 6 in. deep. The trenches were dug up in 6-in. squares of turf and the larvae classified by the number present in the square (sample density). There was no correlation between sample density and weight of larvae, in spite of the fact that some very high sample densities were encountered (up to 16 larvae per 6-in. square). All the samples used in the correlation

were from grass unattacked by birds. A number of 6-in. samples from bare patches was also taken and a total of 112 larvae from these was weighed. The mean weight of larvae from bare patches was 166.2 mg., compared with a mean weight of 180.7 mg. for the 508 larvae above, the difference of 14.5 mg. being highly significant ($P = 0.01$).

TABLE XVI.

The weights of larvae from bare soil, loose turf and firm turf.

Sample	Sample density (no. of larvae per sq. ft.)	Condition of turf	No. of larvae weighed	Mean weight (mg.)	Standard error
A ..	9	bare	7	227.6	12.65
B ..	10	bare	10	230.7	10.16
C ..	23	firm	17	229.6	10.44
D ..	10	firm	8	218.1	8.71
E ..	13	firm	11	225.2	9.00
M ..	14	firm	13	200.8	7.36
L ..	16	loose	13	205.9	10.71
K ..	19	loose	16	206.6	9.04
F ..	9	bare	6	199.2	6.24
G ..	14	loose	12	221.7	12.18
H ..	11	firm	11	202.0	12.22
I ..	9	firm	8	203.0	12.35
N ..	12	bare	7	193.3	17.88
O ..	8	loose	20	208.7	10.30
P ..	16	firm	42	209.8	6.27
Q ..	14	firm	56	211.5	5.29

From this set of observations it seems that, while population density does not affect the growth of the grub at the densities encountered, barring of the soil does make for lighter larvae. Some grubs from the bare patches perhaps migrate to the surrounding, unattacked turf and some are eaten by birds, but it seems likely that the missing grubs will merely represent a random sample of the bare-patch population, as regards weight. Thus the lower mean weight of larvae from bare patches is probably due to the almost total absence of food once the top cover is removed.

The question of the incidence and timing of damage by birds will be discussed in detail by Dr. Milne in a later paper. Here it is enough to say that such damage is not an inevitable concomitant of high population density. A sufficiently high population loosens the vegetation and so makes it possible for the birds to get at the grubs. Once this has happened, however, the appearance of birds on the scene is governed by factors such as scarcity of other foods, etc. Bird damage may occur at different times during the third instar, thus varying the duration of exposure of grubs in bare patches to low food supply. In short, while this appears to be a factor which will tend to reduce growth and hence the mean reproductive rate the following spring, the circumstances, as in all ecological situations, are extremely complex.

The lack of correlation between sample density and weight indicates that a population rarely, if ever, gets short of food except when the top cover is removed. The population sampled in this case was one of the highest encountered in six years' observation in the Lake District. Two experiments on the growth of grubs at different densities were inconclusive and gave no evidence either for or against the idea that a high density produces lighter hibernating grubs and pupae. The

main difficulty encountered in the experiments was that the larval mortality rate increases with increasing density.

It is impossible to maintain a really high-density culture. The reason for this is to be found in the behaviour of the larva. On two occasions, chafer grubs have been found in circumstances suggesting that they had killed and were eating other beetle larvae encountered in soil (Milne, 1956). When two grubs, moving through either loose soil or packed soil, meet and touch, there is an immediate though apparently undirected reaction. The grubs, normally lying in the form of an open U, contract the body sharply, apposing the head and raster. The mandibles open and close vigorously during this movement. Quite often some part of the body of one or both of the grubs gets in the way of the snapping mandibles and is punctured. Such punctures almost invariably become infected and eventually prove fatal. This was the main cause of failure of the density experiments.

The result is interesting as suggesting a mechanism that in nature may limit the population density. Additional evidence for its occurrence and effectiveness comes from the survival figures for Experiment 1 (see Table I). The number of grubs per block throughout the feeding period is shown in fig. 6. At 31 days, density (grubs per block) was decreased by dividing the surviving larvae in the initial block between two blocks, at 99 days it was increased by recombining the larvae in one block (see p. 128). The grubs were in a fairly loose sifted soil which allowed them more freedom of movement than normal turf but which resembled quite closely the conditions just below the surface of completely undercut vegetation. Here the grubs lie in a layer of loose crumbled soil which offers little resistance to movement.

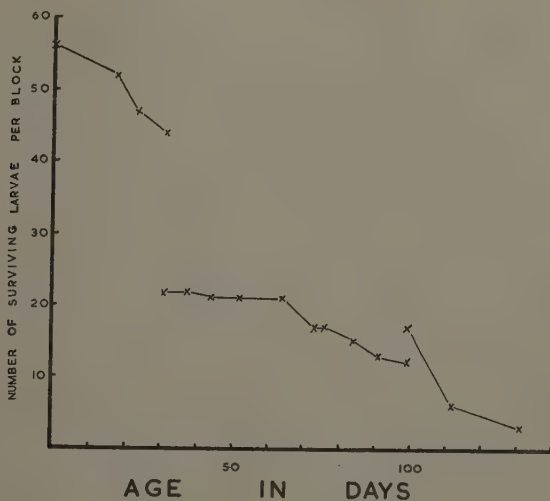


Fig. 6.—Mortality, population density and size of larva.

Examination of fig. 6 indicates that mortality increases with larval size and population density. Thus, up to 31 days the mortality rate (as shown by the slope of the curve) is increasing though, of course, density is decreasing. Again, between 31 and 99 days the numbers fall off slowly at first then more quickly as the grubs increase in size and activity.

The effect of density on survival is seen at 31 days and again at 99 days. At

31 days the density was halved by putting the grubs into two plaster blocks instead of keeping them in one. Survival improved dramatically. At 99 days, on the other hand, density was increased by re-uniting the surviving feeding grubs in one block. Over half the larvae died in the next 13 days. It is most unlikely that these effects were produced by lack of food. The food (germinating grass seeds) was changed each week and there was always plenty left at the changes. The rise and fall of mortality with density and size of larva is quite clear-cut.

Discussion.

In common with most other chafer, *P. horticola* is a catholic feeder and can survive on a variety of plant roots. The larva can even live in culture, on a spaghetti-like proprietary breakfast food, though growth is slow and stops completely after the first moult.

The species is remarkable in having so short a feeding period. Most chafers have a life-cycle of 2-4 years and the larva feeds for a large proportion of that time. Larvae of *P. horticola* feed for only 3-4 months of the year and the food reserve stored up in that time is enough to complete the life-cycle, though adult feeding does play a part (Laughlin, 1956).

At first sight it also seems remarkable that the larvae should be feeding most actively in the autumn, when plant growth is at a minimum and temperatures are falling. However, it has been shown that the stores of organic materials in the roots of grasses are at their highest level at this time of the year. Weinmann (1948) states: "... reserve carbohydrates are, together with nitrogen and mineral elements, translocated during maturation to roots and rhizomes, where they are stored over winter to be re-utilised by the plants in the following spring for the production of new top growth. Underground development in grasses is often most active at times of the year when herbage growth is at a minimum." In general it appears that the amount of reserve substances in grass roots is increasing through the late summer and autumn. In fact, the food value of the roots increases parallel with the growth and requirements of the larvae.

Two features emerging from the work described in this paper may be of importance in nature:

(1) It is frequently stated in the literature that white grubs hibernate with the coming of cold and frosty weather. This is true, but it is usually implied and sometimes stated that the cold weather is the cause of hibernation. While this may be so for other chafers, it certainly is not for *P. horticola*. In Experiment 1 (p. 129) it was found that the larvae hibernated in the normal way though kept throughout the growth period at 15°C. Again, in the experiment (b) described on p. 144, there were several frosts and cold spells in the weeks before the pots were examined, yet the "late" grubs hibernated at least three weeks after the "early" grubs, with no apparent regard for the weather. It has been found in the field also (Milne, 1956) that hibernation is independent of the weather, in other words, that larvae hibernate when full-grown and not before.

(2) It was noted in the experiment (c) (p. 147) that males tended to predominate in samples of hibernating larvae taken early in the change-over period. Barely 10 per cent. of the pupae from the first sample were females but the proportion rose steadily to 50 per cent. in the last sample (see Table XIV). It thus seems probable that, in general, male larvae are the first to finish feeding and burrow down to hibernate.

The ecological significance of these two points lies in the fact that feeding grubs are less protected from frost than are the hibernating grubs down in their earthen cells. Excessive wetness also affects the former more than the latter (see Schaefferberg, 1947). Thus, severe weather conditions during the change-over period will tend to kill chiefly feeding grubs and hence more females than males. This would provide part of the explanation for the variation in sex ratio

found in nature. Males always predominate in field populations of pupae (see Milne, 1956).

The above discussion is a digression from the theme of this paper in that the mechanism suggested affects the reproductive rate of the population as a whole, through the alteration of sex ratio. Variation in growth rate and pupal weight (the main concern of this paper) affects the reproductive rate of the population by way of the egg-laying capacity of the female. However, selective mortality during the change-over period also affects the mean weights of male and female pupae in the population, and hence the mean fecundity, since the heavier individuals hibernate first (see p. 147).

Thus, the population arising from eggs laid towards the end of the flight period is at a double disadvantage at hibernation time. The larvae hatch late and so tend to be light, with the result that fecundity the following spring is low; and death by frost and wet tends to weed out more females than males and reduce still further the egg production *per head of population*.

Summary.

Previous work has shown that, under natural conditions in the Lake District, larvae of *Phyllopertha horticola* (L.) hatch in early July and feed actively on the roots of pasture plants during the next 3½–4 months, undergoing two moults. They then empty the gut and enter hibernation, pupating the following spring. Stores of organic material in grass roots are at their highest level during this autumn feeding period. It has also been shown that the egg production depends almost entirely on the weight of the hibernating larva or of the pupa, which thus plays an important part in determining the reproductive rate of the population, and studies were accordingly made on larval growth and certain factors affecting it.

Newly hatched larvae were cultured at 15°C. in moistened plaster-of-paris containers filled with a mixture of soil and germinating grass seeds. They moulted at about 20 and 45 days after hatching, and stopped feeding and entered hibernation at about 100 days. When the seeds were scattered on the soil surface, larval growth was slower. The mean larval weight, plotted against age, gave a sigmoid curve; in the first instar and most of the second, the rate of increase in weight was proportional to the weight, but thereafter, up to the time of hibernation, it was more or less constant. The rate of growth of the individual larva was irregular, being slower at the moults and variable even in the middle of the instar.

Larvae cultured under semi-natural conditions in pots of growing grass in the open moulted about 3–4 and 7–9 weeks after hatching and entered hibernation at 100–120 days. Growth is possible on a wide variety of food plants, larvae cultured on 13 species of pasture plants grown in pure stands surviving to the hibernation stage on all but two of them.

During hibernation, the larva loses 20–25 per cent. of its weight, mostly in the first few weeks. The pupal weight is almost constant and does not appear to be affected by the temperature treatment of the hibernating larva. It is thus a useful index of effective larval growth.

The mean and (in brackets) range of the weights of all pupae collected in two fields in the Lake District between 1950 and 1953 were 139.3 mg. (65–242) for males and 171.3 mg. (72–310) for females. Field samples of hibernating larvae and of pupae show considerable variation in weight from place to place, from year to year and within apparently homogeneous areas.

Variation in the time at which larval growth takes place is a major cause of variation in pupal weight. The growth period of larvae in a field at Buttermere was three weeks earlier in 1952 than in 1950, though of the same duration, and the resulting pupae in 1953 were heavier than those in 1951. Two lots of larvae of similar parentage, grown in adjacent plots of grass out of doors, one of which both hatched and entered hibernation three weeks before the other, likewise showed a

difference in weight at hibernation, the earlier lot being the heavier. A series of weighed samples of larvae taken from part of a field at Ambleside in 1953 at weekly intervals during the period when they were entering hibernation showed that heavier individuals did so before lighter ones, and males before females. Factors inducing mortality during this period thus operate selectively against females, because these are exposed to them for longer.

Field-collected larvae fed in the third instar on roots of lettuce produced pupae the following spring that were significantly heavier than those from larvae fed on roots of either ryegrass or clover.

There is no evidence to show that population density affects the weight of the hibernating larva or the pupa. On the other hand, larvae from soil from which the damaged turf had been stripped by birds were significantly lighter than those from the surrounding undisturbed sward.

When moving through the soil, larvae may meet and fatally injure each other by an undirected "snapping" reaction. This mechanism may limit population density. In an experiment in which larvae were reared in loose soil on grass roots, the mortality rate was seen to increase with the size and activity of the larvae, and also with the larval density.

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OBSERVATIONS ON CHIRONOMIDAE AT KHARTOUM.

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(PLATE V.)

The history of the Chironomid problem in the Sudan has been described by Lewis (1956a). In the dry season the Blue and White Niles and the main Nile have some of the features of a lake and produce great numbers of Chironomids which are a serious pest at Khartoum and Wadi Halfa in the early part of the year. The working of dams has apparently caused these Chironomids to become a pest, possibly by creating large breeding areas or by altering the general conditions. Although members of the CHIRONOMIDAE are not a pest in most parts of the world they cause intense annoyance at these towns and are probably largely responsible for asthma and hay-fever there.

The present paper describes investigations on the biology of the CHIRONOMIDAE at Khartoum and on protective measures. A complete study would involve years of work on larval and pupal taxonomy, larval behaviour and other features of the insects, and on the hydrobiology of the Nile at various places. This has not been practicable, so the Chironomids have been studied as much as the demands of other problems permitted, and attempts at control, with accompanying observations, have been made in the hope of finding satisfactory measures or at least of adding to our knowledge of the insects.

The following pages contain many references to *Tanytarsus manicus* (Wlk.) and to CHAEBORINAE (CULICIDAE). Very little is known about the biology of Ethiopian CHIRONOMIDAE and that of *T. manicus* is of interest in the present work because *T. lewisi* Freeman, a common Khartoum species, is closely related to it. The larval biology of the CHAEBORINAE, a species of which occurs at Khartoum, throws light on the breeding conditions of the midges there. Furthermore, in some countries species of CHAEBORINAE cause a plague rather like that of Chironomids at Khartoum, particularly in California where they have been successfully controlled (Lindquist, Roth & Walker, 1951).

Observations on Chironomids described below refer to *Tanytarsus* except where otherwise indicated. In Africa, as in Britain (Macan, 1947), the larval and pupal taxonomy of the CHIRONOMIDAE have been little studied. There was no time for detailed taxonomic work at Khartoum and most references to early stages of *Tanytarsus* refer to the genus as a whole.

Methods.

Obtaining eggs and young larvae.

Many thousands of *Tanytarsus* eggs can be obtained in a few minutes. Females are collected with a suction tube from beneath a light attached to a wall sheltered from the wind, and are blown into one or more petri dishes half-full of water which are then covered. Eggs are soon laid and sink to the bottom, and the dishes are then opened and filled so that the floating adults can be poured away. Eggs can also be obtained by trapping midges in a basin of water beneath a light. After hatching, the minute larvae gather at the lighted sides of the petri dishes in milky patches from which they can be removed by pipette if required for testing

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insecticides or for other observations. Some eggs of other genera are also found in the petri dishes as well as parasitic worms and mites.

Collecting larvae and pupae.

Larvae and pupae were obtained by taking river-bottom samples with a small net or, generally, by using a tow-net (Pl. V, fig. 3) to which was normally attached a closed empty bottle so that the net remained near the surface without catching insects drifting on it. The mouth of the net was 30 cm. in diameter and the trailing end led into a metal tube 6.8 cm. wide and 25 cm. long to which were soldered three tubular metal floats each 4 cm. wide and 14 cm. long. To the downstream end of the tube was tied a cloth bag 22 cm. wide and 60 cm. long (when flat) which received the catch without appreciably reducing the flow of water through the net. The floats prevented the tube and bag from dragging down the end of the net in slow water, but let them revolve so that the net did not remain twisted if it became so when placed in position on a windy night in rough water. The bag was removed in due course and placed in a jar of formalin solution, other bags being attached to the net as necessary. The addition of wet bags to the jar soon dilutes the formalin and it is important to replenish it and ensure that the pupae are killed quickly so that subsequent examination will show their condition at the time of capture. At first the net was towed from a boat but later it was usually moored to a buoy to ensure a constant rate of catching. Supplementary observations were made at Wad Medani where the river narrows and flows quickly past a rocky promontory, and here the net was usually attached to a bamboo pole fixed like a large fishing rod (Pl. V, fig. 2).

The plankton obtained was examined in petri dishes under a lens or binocular microscope, and midge larvae and pupae separated from the numerous Crustacea. This is a time-consuming process owing to the small size of the *Tanytarsus*. The volume of the mass of Crustacea was roughly recorded by noting its height in the 23 mm.-wide specimen tubes which were used, and was a convenient check on the proper working of the nets (fig. 8).

Collecting adults.

The number of midges collected by light-trap was estimated by weight (Lewis, Henry & Grindley, 1954) from mid-November onwards when the rains had ceased and the large insects were few enough to be removed.

Few emergence traps were used because time was limited, but one or more of the types mentioned by Mundie (1956) would be useful.

Observations on pupae and emergence times.

It is difficult to observe *Tanytarsus* pupae directly in the field because they are so small and are active mainly in the open river at night, so generally their activities were deduced from the condition of caught specimens.

The species of male pupae could be ascertained by examining the terminalia. The developing coloration of an enclosed male or female adult indicated the ageing of a pupa. Male pupae were classed as dark and old when more than half of each antenna had darkened, and females could be roughly classed according to the appearance of the antennae and other parts. In pupae about to emerge, the film of gas beneath the cuticle, which probably results from the absorption of the moulting fluid (Wigglesworth, 1953), produces a characteristic silvery appearance, and in specimens killed while emerging the abdomen can usually be seen partly withdrawn from the pupal skin. Adults which had emerged in the tow-net were easily distinguished from older ones which had accidentally drowned. In the former the wings were incompletely formed and the abdomen swollen, and in the males the antennal hairs were pressed against the antennae. For estimating emergence times, pupae killed when about to emerge or emerging, and fresh

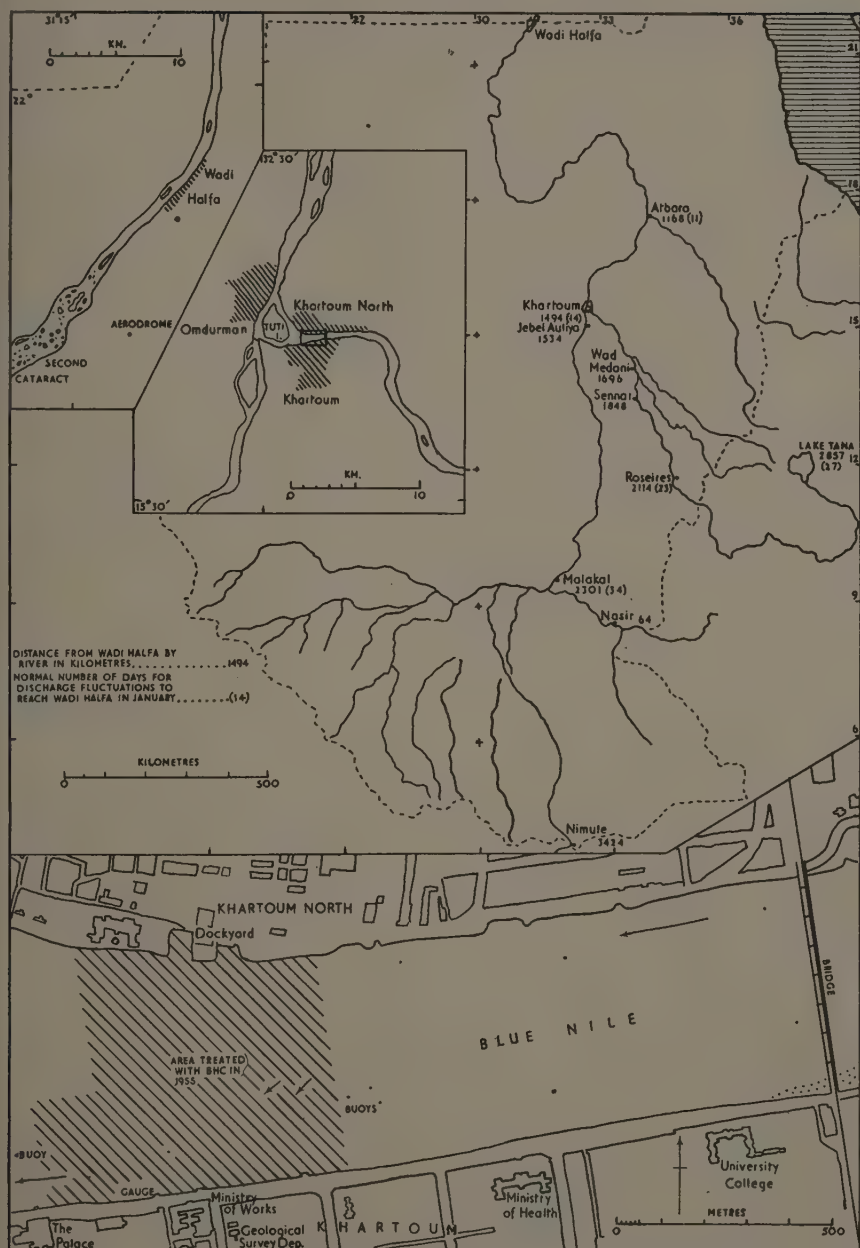


Fig. 1.—The principal rivers of the Sudan, the Khartoum and Wadi Halfa areas, and part of the Blue Nile at Khartoum.

adults, were usually considered as "emerging". In ten-minute catches on 6th April 1952, unlike earlier one-hour catches, more females than males were found to have died while emerging, possibly because they can emerge less easily in the net. The total number of specimens (shown in fig. 7) was 2,061 and the percentages of males and females, respectively, were: total, 49.0, 51.0; silvery and emerging, 1.7, 7.2; not silvery and emerging, 1.8, 4.2; emerged, 14.9, 10.8.

Appressed antennal hairs were also useful, during oiling operations, for distinguishing males which had just emerged and been killed by the oil from others which had fallen on to it. Oiling can thus be used for studying times of emergence.

The sudden appearance of pupal skins in mid-river provides information on the emergence times of various species. Millions of pupal skins often accumulate on a lee shore in a foam-like mass and indicate the prevalence of species. Pupae of *Nilodorum brevibucca* Kieff. seldom appear in tow-net catches and probably rise so quickly that they could be caught in a vertical trap.

Keeping larvae and pupae in captivity.

Tanytarsus larvae which had hatched in the laboratory did not live more than a few days although they were kept in tap water, canal water and river water, and in petri dishes, large glass tanks, an outdoor pool in which other Chironomid larvae were living, and a concrete tank containing flowing aerated water. Malloch (1915, p. 278) referred to the difficulty of keeping some Chironomid larvae alive and recommended mooring a fine gauze rearing cage in a river, but tests on these lines with the microscopic larvae of *T. lewisi* in the Blue Nile were unsuccessful. The pupae of *T. manicus* are reported to be so sensitive to handling that bred pupae are better than captured ones for breeding adults. Pupae of *T. lewisi* are also delicate but if carefully collected in the early morning will survive a few hours till the adults emerge.

The Environment.

Khartoum is on the left bank of the Blue Nile (fig. 1) and has a river frontage of some five kilometres. The annual sequence of river discharges and levels at Khartoum is shown in fig. 2 which is based on data in Egyptian Government publications listed by Lewis (1956a). In August, at the peak of the Blue Nile flood, the river has a discharge of about 550 million cubic metres a day, flows fast and is heavily laden with silt. By November there is little silt (Beam, 1906) and the discharge is much reduced, considerably more so than before the Sennar dam was built, because water is now diverted to fill the reservoir and the irrigation canal (top of fig. 2), and because there were some very high floods early in the century. The White Nile has a bigger November discharge than the Blue Nile and therefore ponds it up at Khartoum and regulates its level for many kilometres upstream. Since 1938, this effect has been increased, from about February to April, by the release of water from the Jebel Auliya dam on the White Nile. At the same time the Blue Nile discharge is slightly increased by water released from the Sennar reservoir. Early in the flood the Blue Nile begins to exceed the White and actually reduces its discharge for a time (fig. 2).

In winter, the channel at Khartoum, previously some 500 metres wide, narrows above the Blue Nile bridge, and most of Khor Tuti (the arm north of Tuti Island) dries up. When the gauge reading is 11 metres, a channel beneath the north end of the bridge is about eight metres deep and there is another deep channel south of Tuti Island. Much of the intervening area is about three or four metres deep and much of the water remaining in Khor Tuti is about two metres deep. The water in the main channel flows at about 700 metres an hour between January and February and there is usually a surface cross-current caused by the north wind. In view of the slowness of the current and Brundin's remarks (1951) on the currents of rivers, the water is believed to be almost still near the boundary layer

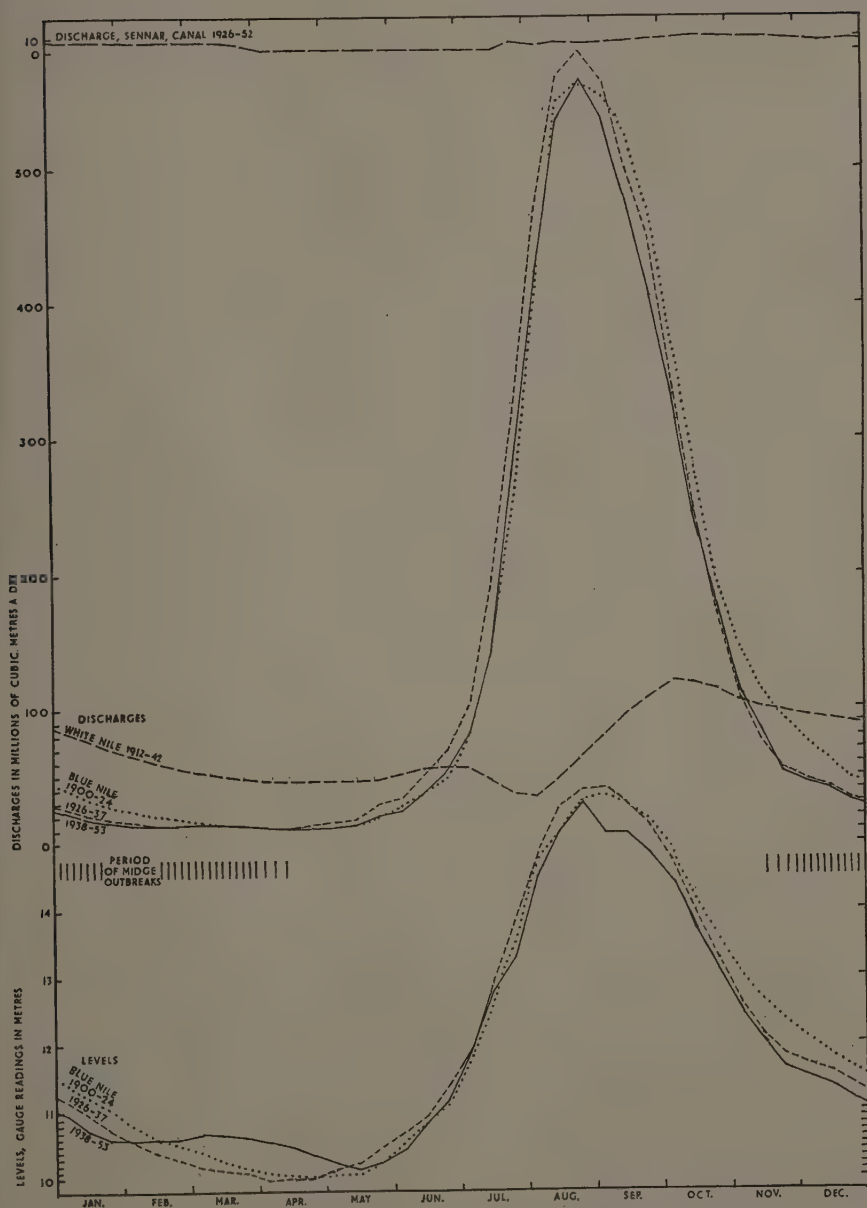


Fig. 2.—The mean discharges, in ten-day periods, of the canal at Sennar, the White Nile at the Mogren point, just before it is joined by the Blue Nile at Khartoum, and the Blue Nile at Khartoum, and the mean levels of the Blue Nile at Khartoum.

at the bottom. The river bottom is undulating and consists of patches of sand and mud.

The winter climate is very dry, with warm days and cool nights. The average daily maximum and minimum temperatures from January to March are 34.5 and 16.7°C. and the average relative humidity at 1400 hr. is 15 per cent. The wind is northerly at this season, and usually diminishes at sunset and increases about two hours after sunrise.

Some herbage grows on the river bank and there are many trees and shady gardens that extend inland for a distance of about 300 metres from the river (see Pl. V, fig. 1). The Blue Nile is practically devoid of macroscopic plants but at times the blue-green alga, *Anabaena*, is so abundant as to make the water look almost solid at night. Rżóska, Brook & Prowse (1955) have shown that phytoplankton and zooplankton appear in November and rise to a peak in February, with a second rise in May and June after an unexplained decrease in March and April.

Few animals other than species of Chironomids are abundant in the river. The following examples, from a one-hour evening tow-net catch from a motor-boat on 3rd February 1951, show the sort of numbers of several forms often seen in the plankton: 3 Mermithid nematodes (probably parasites from the Chironomids), 367 Oligochaet annelids, hundreds of thousands of Copepod Crustacea, 20 Ephemeropteran nymphs, 2 Corixids, a water beetle, 11 *Chaoborus* larvae, 487 Chironomid larvae and 196 pupae, 18 Ceratopogonid larvae and a pupa, 1 young *Simulium* larva, 40 mites and a fish 4 mm. long. Caddis nymphs are not uncommon, and the Branchiuran, *Chonopeltis inermis* Thiele, was found once. Dr. J. P. Harding informs me that this fish parasite was previously known only from Lake Nyasa.

The annelids in the plankton, which increase at dusk, are smaller than some of those found on the bottom.

Adults of the large mayfly, *Polymitarcys savignyi* Pict., were abundant round lights on several evenings in January 1952 and on a few other occasions. Flying Corixids are sometimes numerous in light-trap catches, for example, on several evenings in November 1951.

The Khartoum *Chaoborus* are discussed by Lewis (1956a, 1956b). In the tow-net catches of January 1955 the average number taken in half an hour was 1.7 and the maximum 29, and pupae were occasionally seen. In February the average was only 0.2.

Ceratopogonid larvae are common in the plankton at night (fig. 5) and pupae are seen mainly by day (Table II). In January 1955, up to 35 larvae were seen in a half-hour catch, but there were few in February.

A small species of the Trichopteran genus *Cheumatopsyche* was found in light-traps on 25th March 1951 and several evenings in April, and was common on several other dates. A species of this genus is common on Lake Victoria (Corbet & Tjønneland, 1955).

In addition to the minute fish sometimes caught in tow-nets, some small ones are found to be seen at the surface in the evening and morning when Chironomids are emerging. For example, many, including the voracious *Micralestes acutidens* (Peters), were seen before dawn on 24th January 1953 when adults of *Tanytarsus* were emerging, and *Chelacthiops bibie* (Joannis) was found at 1830 hr. on 17th March 1953 when *Nilodorum brevibucca* was emerging. Others are mentioned in the section on enemies. A useful guide to the fish fauna has been published by Sandon (1950).

Notes on the Species of Chironomidae and the Genus *Tanytarsus*.

At least 26 species of Chironomids occur at Khartoum, as shown in the following list. Mr. Paul Freeman of the British Museum (Natural History) has identified most of these and is studying additional material.



Fig. 9.—The respiratory organs of some pupae: (a) *Procladius* sp.; (b) *Tanypus* sp.; (c) *Nanocladius vitellinus*; (d) probably *Nilodorum brevibucca*; (e) an undetermined species of the CHIRONOMINAE; (f) *Cryptochironomus* sp., probably *aegyptius*, and part of its mesonotal profile; (g) *Cryptochironomus* sp., probably *graminicolor*; (h) *Polypedilum* sp.; (i) *Tanytarsus lewisi*; (j) *Tanytarsus* sp. near *pseudomancus*.

TANYPODINAE	<i>Nilodorum</i>
<i>Pentaneura</i>	<i>brevibucca</i> Kieff.
<i>cygnus</i> (Kieffer)	<i>Cryptochironomus</i>
<i>dusoleili</i> (Goetghebuer)	<i>aegyptius</i> Kieff.
<i>nilotica</i> (Kieff.)	<i>camelus</i> Kieff.
<i>pictipes</i> (Kieff.)	<i>fimbriatus</i> (Kieff.)
<i>Procladius</i>	<i>graminicolor</i> Kieff.
<i>noctivagus</i> (Kieff.)	<i>nilicola</i> Kieff.
<i>Tanypus</i>	<i>Stictochironomus</i>
sp.	<i>caffrarius</i> (Kieff.)
ORTHOCLADIINAE	<i>festivus</i> Kieff.
<i>Cricotopus</i>	<i>Polypedilum</i>
<i>sudanicus</i> Freeman	<i>iris</i> Kieff.
<i>Nanocladius</i>	<i>scotti</i> Freeman
<i>vitellinus</i> Kieff.	New genus (?)
CHIRONOMINAE	sp.
<i>Chironomus</i>	<i>Tanytarsus</i>
sp.	(<i>Tanytarsus</i>)
<i>Dicrolendipes</i>	sp.
<i>cordatus</i> Kieff.	(<i>Cladotanytarsus</i>)
<i>fuscotatus</i> (Kieff.)	<i>lewisii</i> Freeman
	<i>nilicola</i> (Kieff.)
	sp. near <i>pseudomancus</i> Goet.

The taxonomy of the adults of some of these species was discussed by Freeman (1955a, 1956). Although the early stages have not been studied in detail the following key is of some value.

Key for the identification of the pupae of some of the CHIRONOMIDAE
at Khartoum, based on characters of the respiratory organ.

1. Peg-shaped with rounded tip, dark brown *Pentaneura*, *Procladius*
Not shaped thus 2
2. Spheroidal *Tanypus* sp. 3
Not spheroidal 3
3. Without branches apart from minute processes 4
Branching 6
4. Wide and blunt *Tanytarsus* sp. near *pseudomancus* 5
Slender and pointed 5
5. Sigmoidally curved (pupa not very dark) *Tanytarsus lewisii*
Straight and very small (pupa very dark) *Nanocladius vitellinus*
6. With about five leaf-like branches *Polypedilum* sp. 7
With many filamentous branches 7
7. Very long and slender with about 30 branches *Cryptochironomus* sp., possibly *graminicolor*
Bush-like with more than 100 branches 8
8. Very large, about 1.4 mm. from base to tip *Nilodorum brevivucca*
Not very large, not more than 0.8 mm. from base to tip
 Dicrolendipes fuscotatus, *Cryptochironomus* sp. (small,
 common), *Stictochironomus caffrarius*

The numbers of various species change during the season but on the whole the *Cladotanytarsus* species, *T. lewisii*, *T. nilicola*, and the *Cryptochironomus* mentioned in the key, probably *aegyptius*, appear to be the most numerous. The numbers of pupae obtained in certain collections at Khartoum and Wad Medani are shown in Table I. One, two or three species of *Tanytarsus* comprised 81 per cent. of the total, and pupae which were probably *C. aegyptius* 7 per cent.

The medium-size pupae of *Pentaneura* and *Procladius*, which are often seen in the plankton, are very like those of mosquitos, as Goetghebuer (1927) pointed

out. Freeman (1953) figured the dappled wing of *Pentaneura dusoleili* and remarked that this species is widespread and common in Africa.

Tanypus sp. is a medium-size dark midge with dappled wings; its spheroidal pupal respiratory organs are of the type figured by Goetghebuer (1927, 1936b), Johannsen (1937) and Wirth & Stone (1956) for related forms. These

TABLE I.

The identification, as far as possible, of 8,396 pupae collected at Khartoum (K) and Wad Medani (W) at various hours.

	3, 4.ii.51 K	3.iii.52 W	30.iii.52 W	5, 6.iii.52 W	6.iv.52 W	4-31.i.55 K	1-28.ii.55 K
<i>Procladius</i> or <i>Pentaneura</i> spp.	12	120	94	34	8	82	28
<i>Tanypus</i> sp.	40	—	—	5	—	53	71
<i>Cryptochironomus</i> sp., small	3	74	101	8	109	213	89
<i>Cryptochironomus</i> sp., probably <i>graminicolor</i>	—	2	11	6	—	10	9
<i>Polypedilum</i> sp.	—	—	—	—	—	6	14
<i>Tanytarsus lewisi</i> or similar form	142	510	1677	416	1613	1341	1107
<i>Tanytarsus</i> sp. near <i>pseudomancus</i>	—	—	—	—	—	—	4
Other species	—	86	125	90	44	14	25

respiratory organs sometimes become detached but the pupa is still recognisable by its size and grey colour and the presence of spicules on the front of the mesonotum. *Chaoborus* pupae have rather similar respiratory organs but in *Tanypus* they are slightly smaller and pointed and have a finer reticulate surface pattern and the pupa is smaller and lacks a broad paddle.

Chironomus is represented by a specimen bred from an irrigation channel at Burri (east of the bridge) in September 1932.

Dicrotendipes fusconotatus is a common East African species (Freeman, 1955b).

Nilodorum brevivucca is a large pale-brown species with a red larva. It is common and widely distributed in the Ethiopian Region (Freeman, 1954b, 1955b) and has been found to be common at Khartoum in March, particularly in 1953, where it gives rise to complaints of mosquitos for which it is mistaken.

Cryptochironomus aegyptius is a very small midge with a brown thorax and green abdomen, and in general resembles species of *Tanytarsus*, which, however, differ in having hairs on the wings and a slightly different venation and other features. The pupae of *C. aegyptius* are about the same size as those of *Tanytarsus* but of a different, rather yellowish, green and the mesonotum has a small median dorsal spine (fig. 3, f). In January and February 1953, such pupae comprised half of the Chironomid pupae other than *Tanytarsus* and amounted to 22.9 per cent. of the total catch of 3,333. In 1953, this species had taken the place of *T. lewisi* as the commonest species by mid-March.

Cryptochironomus camelus is a medium-sized species with a hump-like projection on its thorax and was recorded from Khartoum by Freeman (1955b).

Stictochironomus cafferarius, according to Freeman (1955b), is easily recognised by its heavily pruinose lateral thoracic stripes, and occurs throughout the midge season at Khartoum.

Polypedium scotti is an easily distinguishable brown species with unmarked wings (Freeman, 1954a).

Before considering the four Khartoum *Tanytarsus* it is instructive to review some features of the genus and of *T. (Cladotanytarsus) mancus* in particular. A larva of *Tanytarsus*, as Johannsen (1937) pointed out, has a rather long antenna with five segments, the basal of which is somewhat curved, about twice the length of the rest combined, and is mounted on a prominence. Bause (1914) has described the early stages of many species. The pupa, like that of other species of Chironomids, becomes, before emergence, a pharate adult within the pupal case, as pointed out by Hinton (1946a, b). For convenience, however, it may be referred to as a pupa until the moment of emergence. Johannsen (1937) and Wesenberg-Lund (1943) stated that the pupal respiratory organ of *Tanytarsus* is simple and usually slender, the tergites usually have small paired patches of spicules, and the anal lobes are fringed with filaments. Descriptions of *T. mancus* were given by Coe (1950), Edwards (1929), Goetghebuer (1928) and Pagast (1931), and the male terminalia were figured by Brundin (1947, 1949) and Goetghebuer (1928). Zavřel (1934) described the larva, Krüger (1938), Thiennemann (1954) and Zavřel (1934) described the pupa, and Hennig (1950, p. 262) referred to descriptions of the early stages. The pupal respiratory organ is stout, slightly curved and blunt and has many minute processes. *T. mancus* is known from the Arctic to the Balkans and from Belgium to European Russia (Thiennemann, 1950), and occurs in the British Isles. Goetghebuer (1936a) found adults near ponds and ditches throughout Belgium. Mr. Freeman informs me that a related form occurs in various parts of Africa.

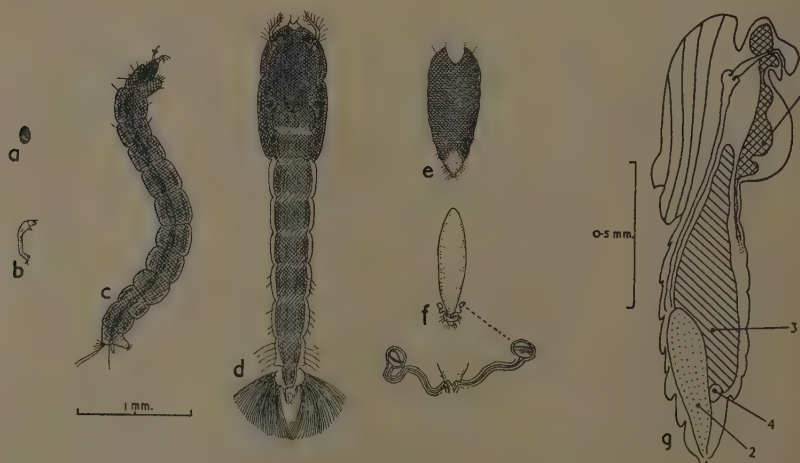


Fig. 4. *Tanytarsus lewisi* or related species: (a) egg; (b) first-stage larva; (c) old larva; (d) pupa (*T. lewisi* ♂); (e) abdomen of ♀ (transparent area shows part of accessory gland not hidden by ovaries); (f) accessory gland and spermathecae with spermatozoa inside; (g) diagrammatic section of ♀ showing (1) large thoracic ganglia, and position of (2) accessory gland (not full), (3) ovaries and (4) spermatheca.

T. (Tanytarsus) sp. is very like the next species, *T. (Cladotanytarsus) lewisi*, but has a darker thorax and, in the male, a darker green abdomen without bands.

T. (Cladotanytarsus) lewisi differs from *T. mancus*, according to Freeman, in being smaller and paler and having slightly different male terminalia. *T. lewisi* is a small pale green species with wings from 1.5 to 1.75 mm. long, reddish scutal stripes and, in the male, dark abdominal bands. The female has a very large accessory gland (fig. 4, e-g) rather like the "gluten gland" of *Chironomus* figured by Miall & Hammond (1900). Each of the two large spermathecae is about 0.057 mm. long, and is transparent so that the long spermatozoa can easily be seen curled inside and often moving rapidly in a circle. The egg (fig. 4, a) is about 0.16 mm. long and 0.10 mm. wide. The newly hatched larva is 0.45 mm. long and its thorax and abdomen are 0.06 and 0.04 mm. wide, respectively. Old larvae are green. The pupa is the same colour and has a narrow thorax so that it looks rather like a larva to the naked eye. Each respiratory organ is small and transparent and has many fine processes, and, unlike that of *T. mancus*, is curved sigmoidally and pointed. In the female pupa the transparent accessory gland is visible near the tip of the abdomen.

A few examples of *T. lewisi* have been found at Khartoum in September and some, with other *Tanytarsus*, from 23rd October onwards. *T. lewisi* is the predominant midge by late December, and, at least in some years, *T. nilicola* occurs in large numbers in February. An apparent diminution of *Tanytarsus* late in February is shown in fig. 8.

T. (C.) nilicola is not unlike *T. lewisi* but has a darker thorax and a grey abdomen which is striped in the male.

The species related to *T. pseudomancus* (a dark species, Freeman, 1955b) is only known from the pupa which is also dark. Few examples have been found, only four, for instance, in the 1955 catch of 3,333 Chironomid pupae.

Chironomids are sometimes found in aircraft arriving at Khartoum. For example, *Chironomus palustris* Kieff. (a widespread species, Freeman, 1955b) and *Nilodorum* sp. were taken in an aeroplane from the south in October 1938, and another *Chironomus* in an aeroplane from Asmara in September 1936.

The Biology of some Chironomidae.

The egg.

Eggs laid in jars by captive females sink immediately, singly or in small clusters, looking like miniature snow-flakes, and most of them adhere to the bottom of the jar. They hatch after two or three days. In nature, most eggs are probably laid on the surface of the Nile.

Breeding places.

The breeding habits of *Tanytarsus* were described by Allee & Schmidt (1951), Brundin (1949, pp. 873, 880; 1951), Carpenter (1928), Goetghebuer (1928), Humphries (1938), Keilin (1944), Krüger (1938), Thienemann (1950, 1954) and Wesenberg-Lund (1943), and those of *T. mancus* by Brundin (1949), Humphries (1938), Krüger (1938) and Thienemann (1950). Larvae of some species inhabit crannies among stones in streams, and others are dominant in oligotrophic lakes, although some can live in eutrophic lakes. The larvae construct tubular protective cases, stationary or moveable, from various materials cemented by salivary secretion. Larvae of *T. mancus* have been found at depths of from 50 cm. to five metres in bottom detritus or sand. They are very numerous in some European lakes, particularly in summer. This was the main species of *Cladotanytarsus* in one lake where up to 3,000 larvae of the subgenus were found to the square metre.

Collections of pupae at Khartoum indicate that *T. lewisi* and other *Tanytarsus* breed in vast numbers in the Blue Nile. They seem to occur in the main stream

rather than in shallow backwaters like Khor Tuti, and many drift with the current at night so that the whole breeding area must be very extensive. Some pupae may be pumped from the river in irrigation water and emerge in tanks and gardens, but the number is probably insignificant.

Habits of the larvae.

Newly hatched larvae of *T. lewisi*, kept in a jar, swim or climb towards the light. Many larvae and pupae of *Tanytarsus* and other Chironomids, and other organisms, rise to the sub-surface layer of the river about dusk and are to be found there during the night. On 5th January 1950, for instance, when the sun set at 1730 hr., five-minute catches from a rowing-boat in Khor Tuti gave the following results at 1732, 1745, 1752, 1801, 1815, 1831, 1845 and 1900 hr., respectively: Chironomid larvae, 0, 0, 51, 85, 99, 297, 140, 170; all Chironomid pupae, 3, 2, 4, 0, 16, 47, 71, 45; *Tanytus* pupae, 0, 0, 0, 0, 12, 29, 45, 25; *Tanytarsus* pupae, 0, 2, 0, 2, 1, 4, 4, 0; Ceratopogonid larvae, 0, 0, 0, 1, 19, 341, 65, 29 (pupae, 1, 0, 2, 0, 0, 0, 0, 1); mites, 0, 0, 1, 0, 0, 10, 5, 3.

Another example was given by five-minute catches made from a slow motor-launch near the Palace from 1800 hr. till 2030 hr. on 3rd February 1951 (when the sun set at 1748 hr.) and continued on the next night. The numbers of Chironomid larvae and *Tanytarsus* pupae, respectively, were: 1805 hr., 1, 1; 1820, 0, 0; 1835, 0, 3; 1850, 10, 7; 1905, 26, 8; 1920, 30, 7; 1935, 14, 8; 1950 to 2405 (average), 23, 6.

On 3rd and 4th February 1951, when 142 *Tanytarsus* pupae were collected at night in the main channel, 487 larvae, mostly of this genus, were also found. In half-hour catches in January and February 1955 up to 70 larvae were found in a catch. Drifting larvae are also seen at Wad Medani, most or all of them at night, but fewer than at Khartoum. On 6th and 7th March 1952, for instance, only 123 Chironomid larvae were found in a catch containing 416 *Tanytarsus* pupae. The nocturnal rise, which must cause the larvae to drift a considerable distance, has been reported in other species by Malloch (1915, p. 284), and Keilin (1944) stated that some Chironomid larvae swim freely. Brundin (1951) considered that, in the slowly moving water of a river which he investigated, small

TABLE II.

Specimens collected by day and night in a 24-hour tow-net catch at
Wad Medani on 3rd and 4th March 1952.

	0600 to 1800 hr.	1800 to 0600 hr.
<i>Procladius</i> or <i>Pentaneura</i> , pupae	0	60
<i>Cryptochironomus</i> , small species, pupa	3	69
<i>Tanytarsus lewisi</i> or species with similar pupa	159	351
The same, pale	20	193
The same, "emerging"	90	77
Other Chironomid pupae	6	86
Chironomid larvae	0	27
Ceratopogonid larvae	1	74
Ceratopogonid pupae	11	0

larvae (including young *Tanytarsus* little more than 0.5 mm. long) were particularly affected by lack of oxygen because they were only in contact with the lowest layer of water. Perhaps the nocturnal ascent of larvae is associated with oxygen deficiency which is presumably more pronounced in the slow water of Khartoum than at Wad Medani. If each larva drifts all night, which is not certain, it is likely to travel eight kilometres or more in 12 hours. The speed of the river in the Chironomid season is therefore of considerable interest and is approximately indicated in fig. 1 by the times taken for fluctuations in discharge to reach Wadi Halfa from various places in January.

The larvae of *T. lewisi* are presumed to feed like the species studied by Walshe (1951) which spin a filter and catch particles for food and case-building.

The duration of the larval stage is unknown. Many adults appear in Khartoum about a month after the Blue Nile has become clear, but their larvae may have started life in the Sennar reservoir during the flood period.

Pupation.

Most pupae collected at any one time appear to be about the same age, and many young, pale ones are seen at dusk, but few in the morning. It is thought that most larvae pupate in the afternoon.

Males comprised 42.8 per cent. of 4,458 *Tanytarsus* pupae collected at Khartoum, and the proportion varied somewhat in different catches.

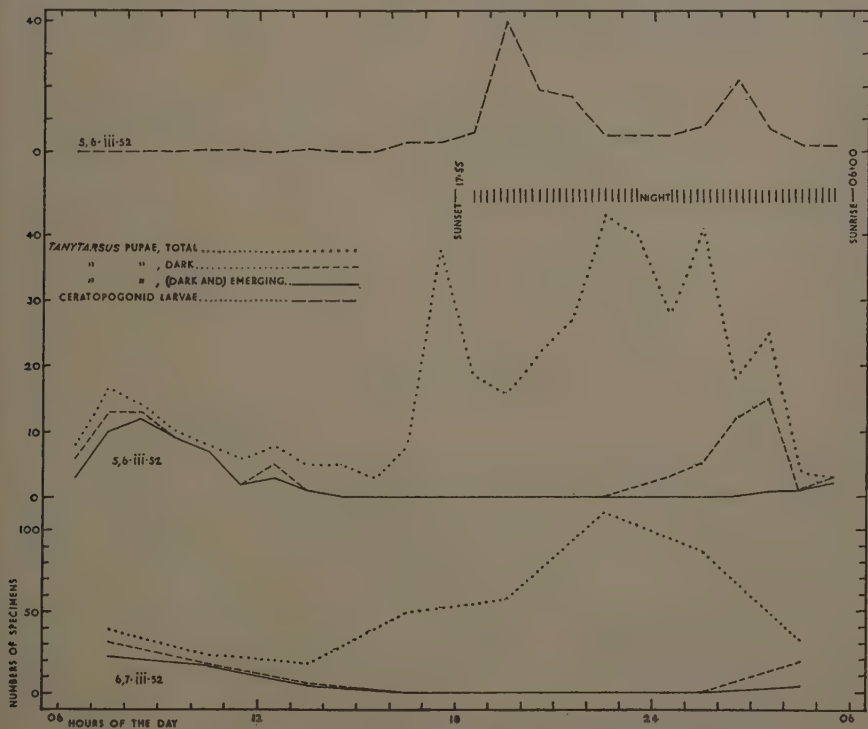


Fig. 5.—Results of 24-hour tow-net catches at hourly and three-hourly intervals at Wad Medani. Each point on the graphs is placed at the middle of the period to which it relates.

Vertical movements of pupae.

The examination of several samples from the river bottom has shown the presence of pupae there during the day. On 22nd March 1955, for example, 994 Chironomid larvae, 12 pupae of *Cryptochironomus* sp., 1 of *Cryptochironomus* sp. probably *C. graminicolor*, 15 of *T. lewisi* and related species, and 3 of *Tanytarsus* sp. near *pseudomancus* were found in six bottom samples.

The rise of many *Tanytarsus* pupae to the sub-surface layer has already been mentioned. The numbers of pupae of various Chironomids in typical day and night catches are shown in Table II.

If *Tanytarsus* pupae are collected at night and placed in a basin of water, they can be seen to swim rapidly and to rise from the bottom of the basin from time to time. It is not known if each pupa normally floats all night. The nocturnal rise

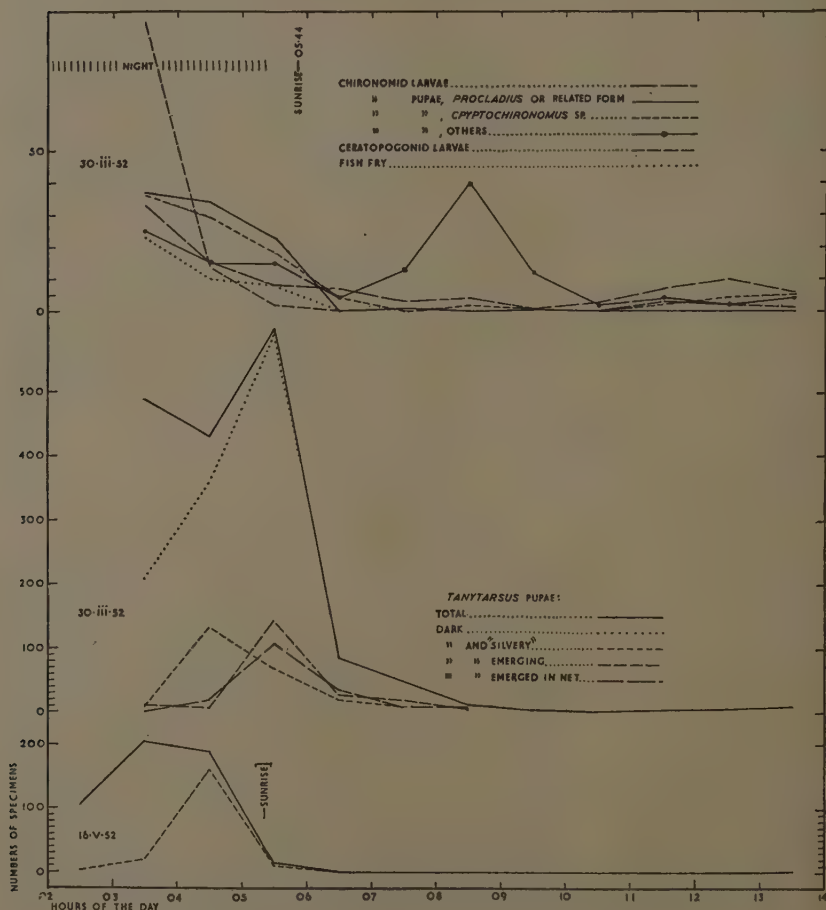


Fig. 6.—Results of two morning tow-net catches at Wad Medani.

of pupae, and a minor secondary rise in the morning when some adults emerge, are shown in figs. 5-7.

That the number of pupae in the sub-surface layers varies considerably on different evenings is shown in fig. 8. Increases in pupae of *Tanytarsus* and other genera apparently tend to coincide roughly with increases in the amount of sub-surface plankton in general and to be associated to some extent with warm weather. Wind and waves seem to have no effect. The figure also shows little variation from one part of the main channel to another, and no regular increase or decrease during the hour after dusk.

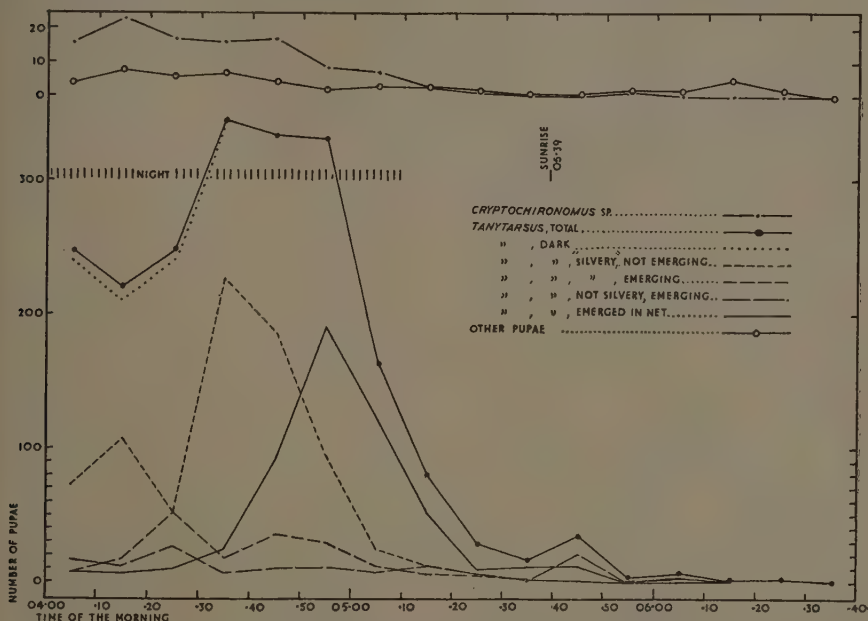


Fig. 7.—Results of tow-net catches at ten-minute intervals at Wad Medani on 6th April 1952.

The darkening of the pupa.

About midnight (figs. 5 & 6) almost all pupae begin to show dark markings due to the developing coloration of the antennae, legs and some other parts of the adult. Other changes are the enlargement of the ovaries and, just before emergence, the appearance of sub-cuticular gas. Nearly all pupae darken about the same time so that one could, by examining a collection of them, tell approximately when it was made. The few pupae found floating during the forenoon are a mixture of young and old, and of those in the afternoon most or all are young, evidently the forerunners of a new lot of pupae after the emergence of those of the previous night. For instance, on 30th March 1952, the numbers of young and old, too small to be shown in fig. 6, were, respectively: 0900 hr., 0, 11; 1000, 0, 4; 1100, 1, 1; 1200, 2, 2; 1300, 6, 0; 1400, 6, 0.

The time of emergence.

The minute midges do not form a conspicuous cloud when they emerge. Large numbers appear along the river bank at dusk and it seemed at first that there

might be a mass emergence at this time, but it is now known that most of the midges have been resting in nearby vegetation. The nocturnal darkening of the pupa gave a clue to the time of mass emergence which is usually the early morning. Captured pupae were seen to emerge at this time, and many were seen emerging from the river at Khartoum between 0445 and 0530 hr. on 24th January 1953 (sunrise 0621) when many could be scooped from the water with a small plate. Similar observations were made at Wad Medani between 0500 and 0530 on 5th April 1952, and other occasions are illustrated in figs. 5 to 7. These show that "silvery" pupae appear some time before most adults emerge, and that a few midges emerge later in the morning. On 30th March 1952, for instance (fig. 6), the numbers of silvery, emerging and emerged individuals at this time were, respectively: 0900 hr., 6, 4, 1; 1000, 0, 3, 1; 1100, 1, 0, 0; 1200, 1, 1, 0. On the

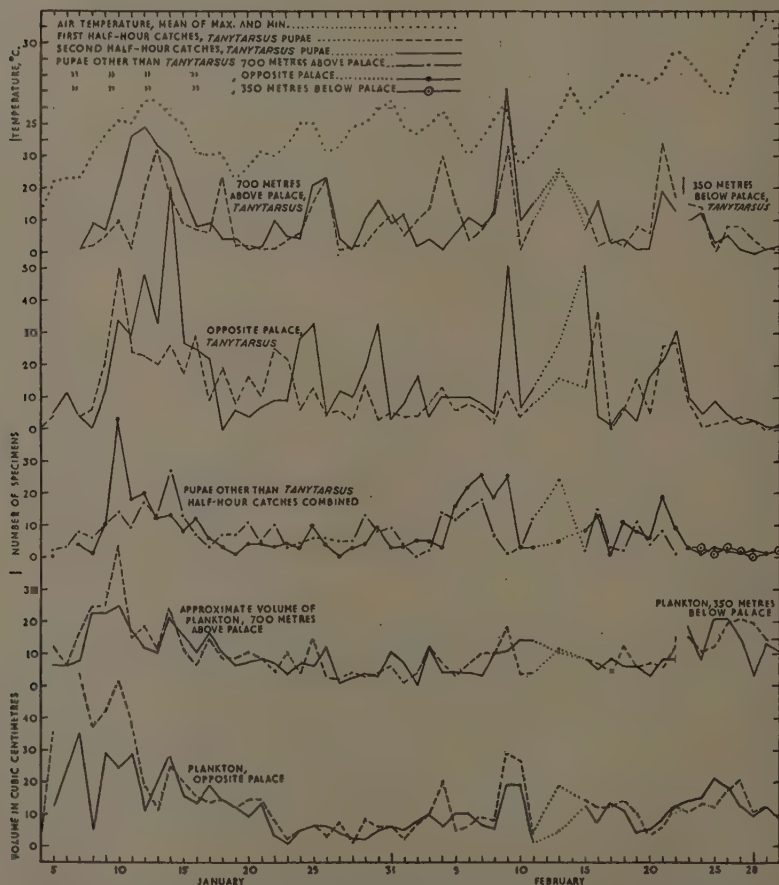


Fig. 8.—Results of half-hour evening tow-net catches of pupae of *Tanytarsus* and other Chironomids at Khartoum in January and February 1955. Two consecutive catches were made each evening, the first starting half an hour after sunset.

same day most of the specimens of other genera caught at 0800 and 0900 had emerged in the net. Males and females emerge at about the same time.

Sprules (1947) studied the time of emergence of aquatic insects in general and concluded that temperature determined the development of pupae by a particular day and that emergence took place when the next period of reduced light intensity stimulated activity. Scott (1936) pointed out that many insects emerged at a particular time of day which, in several cases, was the time of greatest activity of the adults, and Scott & Opdyke (1941) found that most Diptera emerged in the evening and morning. Miller (1941) studied the emergence of Chironomids in Canada and showed that most of those living above the thermocline emerged between 0400 and 0700 hr., when light intensity and temperature were low, and that those from below the thermocline emerged throughout the day and sometimes at night, possibly because they live at a constant temperature in a dim light. Phillipp (1938) found that a species of *Chironomus* nearly always emerged by day. Bates (1949) suggested that the emergence time of mosquitos is determined by the time at which pupation occurs. Nielsen & Haeger (1954) found that, whereas the pupal period of a species of *Aedes*, which they studied, depended solely on temperature, the time of maximum pupation was determined by the distribution of light and darkness, 83 per cent. of larvae pupating from 15 to 24 hours after experimental midnight and 48 per cent. in the period just after sunset.

It is thought that the time of emergence of *Tanytarsus* at Khartoum is not determined by light changes at that time, because there is no mass emergence at dusk and because some adults emerge well before or after dawn. The fact that emergence follows the darkening of the pupa suggests that it occurs as soon as the normal period of pupal development is over and, only by chance, more or less coincides with the dawn.

It was thought that the warmth of summer night, by shortening the pupal period, cause adults to emerge earlier. This does not appear to happen and it may be that the time of pupation is affected by light changes, as was found by Nielsen & Haeger (*loc. cit.*) in a mosquito, and that later sunsets cause larvae to pupate later and so compensate for any shortening of the pupal period.

Pupae of some other genera, like the common *Cryptochironomus*, are pale at dusk, and of others, like *Polypedilum* sp., are dark. The latter may emerge in the evening, like *N. brevibucca* and several others.

Length of the pupal stage.

The pupal stage of *T. mancus* is reported to last only a few hours and the same is evidently true of *T. lewisi*. The above-mentioned observations indicate that it lasts for much less than 24 hours, possibly only 11 or 12.

The process of emergence.

The emergence of mosquitos has been described by Bates (1949) and Howard, Dyar & Knab (1912), and the process is very similar in *T. lewisi* but much quicker. This rapidity is noticeable also in the larger species at Khartoum and is common in the CHIRONOMIDAE according to Malloch (1915, pp. 277, 278) who remarked that adults emerge in a few seconds, so soon after the pupae reach the surface that few fresh adults can be caught on it.

The opened thoracic portion of the diaphanous pupal pelt is a minute bowl strengthened by the cuticle of the median part of the mesonotum, and serves the young fly, for a few moments, as an efficient raft which is presumably very necessary when the water is rough. The strength of the "raft" is demonstrated when thousands of them drift to the bank and are pressed together there by wind and waves. The rafts resist the pressure and look like countless pores in the surrounding scum. The raft has an aquefuge surface, the effect of which is particularly noticeable in *N. brevibucca*, the pelts of which collect in rows

attached by their thoracic portions with the abdomens trailing to one side or the other.

The adult midge takes a few steps on the water before flying away. Few adults are to be seen at the time of emergence and it is supposed that they fly upwards and out of sight.

Flight.

If the adults do not encounter vegetation they drift on the wind far over open country. Many have been seen, for example, at the Gezira Research Farm, Wad Medani, 2 km. from the Blue Nile, and at the Wadi Halfa aerodrome which is 4 km. across open desert from the nearest part of the Second Cataract and much farther from the probable breeding place to windward. The importance of wind is illustrated by the lack of midges in most of Khartoum North and Omdurman in contrast to Khartoum which is to leeward of the breeding area.

The males of *T. lewisi* swarm near the ground, like other very small Chironomids (Gibson, 1945), and are often seen dancing in the afternoon, over sun-lit areas or pale objects, in groups which vary from less than ten to several hundreds. They are often seen over pale objects such as areas of cement, white lines on tennis courts, and table-cloths, and will fly over and follow people swimming. The males are sensitive to sound and dart quickly upward at the sound of high notes. Various sounds, such as carpet-beating 50 metres away, and hammering, have a visible effect. Mating pairs are to be seen moving above or below swarms in the late afternoon.

The midges become very active at dusk, presumably owing to the reduced light intensity. During the total eclipse of the sun on 25th February 1952, which began at 1110 hr. and lasted 3 minutes 9.5 seconds, the writer and other observers in different parts of Khartoum noticed that small Chironomids suddenly appeared when the preliminary darkening of the sky was intensified as the sun disappeared from view. This observation may be comparable to those of Nielsen & Greve (1950, p. 255) in cloudy weather, and Wheeler & others (1932) and Weber (1952) during eclipses.

After dark, midges gather in great numbers round lights, a strong one of which can attract several million. Many individuals appear as streaks of light as they fly quickly about, and some look like pot-hooks because they accelerate for only a short distance. A characteristic feature of large assemblies at lights away from walls is a downward tongue-like extension of thousands of midges for several feet below the main mass. Each individual of *T. lewisi* has a wing area of slightly more than 1 sq. mm., but many thousands of midges concentrated near the light combine to produce a considerable downward draught which blows many other midges downward. The draught can be demonstrated with insecticidal fog which turns sharply downwards on reaching the light but ceases to do so as soon as the midges are killed. A puff of wind disperses the tongue-like extension which re-forms in still air.

Haunts of the adults.

The midges gather in the lee of trees and buildings and are seldom seen far from vegetation in which many of them rest by day. In calm weather they are numerous within about 50 metres of the river. In windy weather they are abundant about 100 metres inland, particularly where there are large wind-breaks in the shape of big buildings or much shrubbery or trees, such as *Citrus*, with dense foliage. Midges are often troublesome up to about 300 metres from the river, that is, to the southern edge of the northernmost blocks of houses. Beyond this they can be annoying at times near the railway station, 1.7 km. inland, and occasionally even farther, according to the wind. On calm evenings Chironomids are numerous on the river bank at Khartoum North.

Length of adult life.

The adults of the Khartoum species of *Tanytarsus*, like those of other Chironomids, apparently do not feed. These delicate flies occur at a time when the air is extremely dry and often hot, and it is believed that most of them only live about 14 hours, that is, from the time of emergence till they drop exhausted below the lights about two hours after dark, or rather earlier in hot weather. Heaps of dead midges are found beneath the lights in the morning. Most adults captured at light and kept in jars die before daybreak. The observations of Lewis, Henry & Grindley (1954) on changes in numbers showed no lag from day to day such as would have been expected if the midges survived more than 24 hours.

Egg-laying.

The larvae and pupae of some species of the CHIRONOMIDAE lay eggs parthenogenetically and display paedogenesis (Wigglesworth, 1953, p. 486), and Wesenberg-Lund (1943) stated that reproduction by the pupa may be an abnormal occurrence in a species which normally carries ripe eggs in the pupal stage. The oöcytes of *T. lewisi* develop in the pupa, and the 240 or so eggs and the large accessory gland (fig. 4, e-g) nearly fill the abdomen of the newly emerged female. No pupa has been seen to lay eggs but no doubt the female can do so very shortly after emerging.

When the river was oiled in the late afternoon many adults, which had presumably been sheltering in vegetation on the right (windward) bank, alighted on the water and were trapped and revealed by the oil. It is thought that many eggs are laid in the afternoon by such adults which have not been blown to leeward inland. Most females collected round lights are found to be fertilised and gravid but would almost certainly have died without reaching the river to lay eggs.

The accessory gland contains a clear fluid most of which evaporates on exposure to air, leaving a clear brittle residue. If the gland is pierced under water the escaping fluid quickly forms a large, rigid, slightly sticky jelly. In most Chironomids all the eggs of one female are surrounded by a single gelatinous covering (Malloch, 1915) produced by the "gluten gland" of Miall & Hammond (1900), but in *T. lewisi* this does not happen and the function of the fluid from the large gland is unknown. Perhaps it forms a thin protective covering round each egg.

Changes in numbers.

In Europe, a succession of generations determines the times of appearance of some species, and in Africa it is thought that the development of a large number of adults on Lake Victoria determines the time of a succeeding outbreak there (East Africa High Commission, 1952). At Khartoum, the clarity of the Blue Nile in October probably enables micro-organisms to grow in the moving water and on the mud and to nourish the larvae of the Chironomids which are abundant by November or December. It is roughly estimated, from observations on pupal pelts and on adults around lights, that several hundred million Chironomids can emerge from the river at Khartoum in one night during a bad season. The midge season ends about March or April, and it may be that the hot weather kills the adults before the evening following emergence. Pupae have been found at Wad Medani in May, and it seems likely that breeding continues at both places till the flood begins in June.

Enemies.

Some adults are parasitised by Mermithid nematodes. One of these was 17 mm. long, and eight others, each about 5 mm. long, were found in a female *Tanytarsus* at Wad Medani.

Tanytarsus pupae are sometimes found in the grip of *Chaoborus* larvae in the net, and many adult midges are attacked by mites.

Chironomid larvae are well known to be an important part of the food of certain fishes, and this is true of Lake Victoria where *Mormyrus* feeds almost exclusively on them (East Africa High Commission, 1951, 1952; Macdonald, 1956). Pekkola (1919) noted that species of *Mormyrus* at Khartoum, where two are common, eat Chironomid larvae, and he mentioned two other fish which do so. Sandon & Al Tayib (1953) examined fish from Khartoum and from up the White Nile, and, although they were unable to dissect all the species present, found Chironomid larvae in no less than 17. One was a young individual of *Alestes nurse* (Rüppel) feeding on Chironomid larvae and pupae at the river surface after dusk. The present writer has found larvae in a member of the family BAGRIDAE, and in a 44 mm.-long specimen of *Barilius loati* Boulenger, caught at the surface at night, and a pupa of *Pentaneura* or *Procladius* in a small individual of *Chelaethiops bibic* taken at the surface before dawn on 24th January 1953. Altogether, 19 species of fish are known to feed at least partly on Chironomids in the Nile but of course vast numbers of the midges escape destruction.

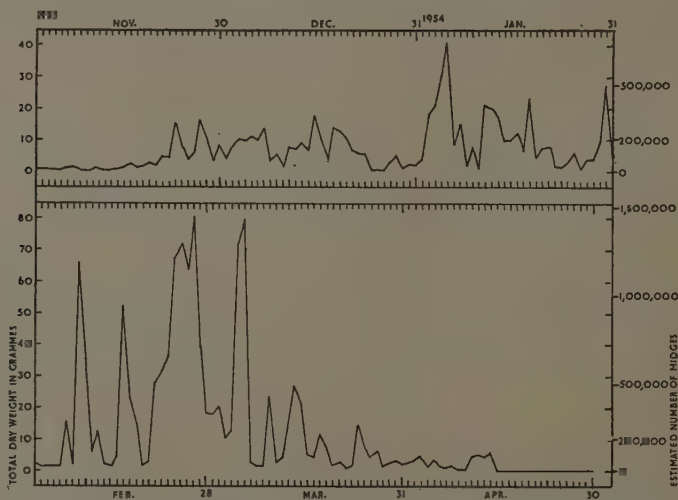


Fig. 9.—The total dry weights of small midges caught during the 1953-1954 season in the light-trap described by Lewis, Henry & Grindley (1954). The graph indicates the number of bad evenings (with more than about 200,000 midges) about 100 metres from the river.

Practical Importance.

Annoyance.

Chironomids cause great annoyance in Khartoum for about two hours after dusk, principally around houses near the Blue Nile. The numbers vary from evening to evening, and fig. 9 and the figures of Lewis, Henry & Grindley (1954) show the number of bad evenings in three seasons. The midges hinder or prevent both work and recreation. The Council of Ministers' Building, the River Hospital and the University College Library are among the important affected buildings in which work is done in the evening. In private houses many people are obliged to sit idly in partial darkness instead of enjoying recreation after the day's work, and some even leave their houses from dusk till the midges disappear. The midge

season coincides with the relatively cool weather when people are recovering from the long hot summer. The Chironomids interfere with cooking and become mixed with food and drink, and soil clothing with their crushed bodies.

On ferry launches crossing the river all lights except the navigation lights sometimes have to be extinguished to enable the mechanics to see. At Khartoum North dockyard the midges spoil new paint on steamers. Bertlin & Olivier (1954) have described a somewhat comparable problem on Lake Victoria where the dead "lake-flies" (in this case Ephemeroptera, although the name is usually applied to Chironomids and Chaoborids), which emit a fish-like smell, eventually corrode all except cellulose paint.

When *N. brevibucca* is common it appears round lights on both banks of the river and can be troublesome at riverside houses at Khartoum North. These do not face the north wind and have no screen of vegetation, and their lights attract these large midges directly from the river after the evening emergence.

Conditions due to allergy.

Many people suffer from coryza after a few years' residence in Khartoum, and some are affected by asthma and other conditions, all of which are generally believed to be largely caused by sensitivity to the Chironomids. The conditions are prevalent mainly in the midge season, and people are affected when they enter the Chironomid area and recover if they leave it. Several people living in valuable river-side houses have found relief only after moving to less attractive areas inland. Among people affected by asthma are some of the University College students and some of the patients at the River Hospital, particularly on the south side of the latter where midges gather in the lee of the building. These patients are moved to the north side for relief.

Attacks of complaints due to this allergy often occur when a midge touches a person's eye or when dust from dead powdered midges is inhaled. Dr. R. Kirk has obtained many positive results at Wadi Halfa in skin tests with antigens prepared from *Tanytarsus* (Lewis, 1956a).

Chaoborus astictopus Dyar & Shann., the Clear Lake Gnat, which is discussed by Lindquist, Roth & Walker (1951), was a serious pest in California and allergy to it is said to have developed. Edwards (1930) recorded *Chaoborus pallidipes* Theo. in great numbers from a place on Lake Victoria, and referred to a fly, probably *Chaoborus*, which appears in vast numbers on Lake Nyasa and is eaten by the people there. Carpenter (1920, pp. 317, 318) mentioned a small fly (perhaps *Chaoborus*) which was commonly said to produce outbreaks of catarrh, possibly of the nature of hay fever, among the European inhabitants of Entebbe. *Chaoborus anomalus* Edw. is sometimes attracted to light with Chironomids at Khartoum (Lewis, 1956a) but is scarcely common enough to cause much annoyance.

Protective Measures.

Chironomids have been controlled by anti-larval measures in various parts of the world, but the scale of operations was much less than what would be necessary at Khartoum. *Chaoborus* has been controlled at Clear Lake by applying TDE emulsion from surface craft (Lindquist, Roth & Walker, 1951), but the large scale operations necessary to cover the 41,600 acres of the lake were evidently extremely costly.

It is likely that radical control of Chironomids at Khartoum would be possible but extremely difficult and prohibitively expensive and would have to be repeated yearly. The river discharge is more than 10 million cubic metres a day and millions of pupae drift down from the upper reaches each night. Anti-larval measures would therefore have to extend a considerable distance above Khartoum. The pupae which arrive there may have travelled eight kilometres or more, and

the larvae probably originated much further upstream. Each test of an insecticide must be a large-scale time-consuming field trial because it is difficult or impossible to keep larvae in captivity. Thorough Chironomid control would amount to an alteration of the fauna of the Blue Nile at a great cost in trained staff, insecticides and boats. Furthermore, wholesale destruction of Chironomids might have an adverse effect on the fish which feed on them.

Of the various measures discussed below it is considered that vacation of houses and of offices along the river front and the use of a barrier of vegetation are the most hopeful. For those who must live or work at night near the river, clearing of some vegetation, air conditioning, or nightly insecticidal fogging would be useful.

Alteration of the flow of the river.

As the Sennar dam has probably been the indirect cause of the midge plagues, it is likely, in theory, that the dam could be used to make conditions unfavourable to the midges. The requirements of irrigation would, however, probably make this impossible. In theory also, it might be possible to use the Jebel Auliya dam to bank up the Blue Nile at night and prevent or reduce the drift of pupae.

Larvicides.

Some exploratory experiments with larvicides were carried out. Expense was a primary consideration in selecting larvicides for trial. Emulsions to permeate the water and heavy oil solutions to cover the bottom were ruled out for this reason, and it was hoped that an insecticide simply scattered on the bottom would be effective.

Laboratory tests.—Such tests were of the nature of a few simple preliminary trials because only first-stage larvae were available. These were placed in petri dishes of water to which powdered insecticide was added at the rate of 0.1 gm. γ BHC or 1.0 gm. DDT per square metre. All larvae were obviously affected after 30 minutes and dead after 24 hours, although controls showed normal activity after these periods. When larvae were exposed to a tenth of these dosages all were affected after 30 minutes and, after 24 hours, BHC had killed many and DDT all. Granular insecticide was then placed in petri dishes to see if it would dissolve gradually and kill larvae a few centimetres away. BHC was effective but DDT was not.

Crude, granular BHC containing 13 per cent. γ isomer and commercial 75 per cent. DDT were placed in large jars of water 28 cm. deep at the rate of 0.1 gm. γ BHC or 1.0 gm. DDT per sq. metre. Three days later, water from the jars was poured into petri dishes in which larvae were placed and examined a day later. In this test, and a repetition of it, all or most of the larvae exposed to BHC were killed, and the controls and those exposed to DDT survived.

Field tests with dusts.—BHC in the form of "Agrocide 7" (2.5% γ isomer) mixed with water was applied with a syringe to still water, 25 cm. deep, in Khor Tuti at the rate of 0.025 gm. γ isomer per sq. metre, and an hour later dead Chironomid larvae were found. Six hundred square metres of deeper water were treated at the rate of 0.004 gm. γ isomer per sq. metre and many dead larvae were found two days later, but higher dosages for shorter periods seemed to give little or no result.

Tanytarsus larvae ingest such small particles, most of them less than 10 microns across, that any wetted dust fine enough to act as a stomach poison would sink too slowly for practical use, particularly in a river, where there must be some turbulence. Even an ordinary coarse dust might contain many fine particles, so it was decided to try a granular insecticide. Granular formulations have already been used on land to prevent loss from wind drift (Farrar, 1953) and in water against mosquito larvae in dense vegetation.

Field tests with granular larvicide.—A granular larvicide for the present purpose should preferably be just coarse enough to sink about four metres in a

few minutes and fine enough to spread well on the bottom. Such a material would probably have to be specially prepared, however, and would therefore be expensive. Even with this it would scarcely be possible for every particle to touch a larva, so the insecticide should be soluble enough to kill larvae in its vicinity and toxic enough to do so in the short time that may elapse after a drifting larva has sunk to the bottom and before it pupates. The slowly-dissolving granules should persist for about four months to obviate the expense of repeated applications. It was hoped that the water at the boundary layer would be stationary and retain BHC in solution.

Crude, granular, 13 per cent. γ BHC (D. 919) seemed worth trying on a large scale although it is rather coarse. It cost £119 a ton—before shipment—in 1955, and was cheaper than other BHC preparations which are formulated from it. It sinks quickly and takes months to dissolve away, and it killed larvae in the preliminary laboratory tests mentioned above. After several methods of application had been tried, a fast motor-launch (Pl. V, fig. 4) was fitted with a wooden stand bearing a small metal drum rotated by a handle, and behind it a series of rollers and another drum projecting over the stern. A continuous conveyor-belt made of deck-chair canvas was fixed over the drums and rollers, and the BHC was wetted (to prevent the dust from blowing into the operators' eyes) and placed on the belt. The launch moved across the river and the larvicide was discharged over the stern and scattered by the wash from the propellor. The launch was steered towards a mark on shore, and the slightly curved course due to the current did not matter because it was repeated at subsequent crossings which were made at intervals of five metres. The selected dosage was heavy, 2 gm. of γ BHC per square metre of the river bottom, so that no larva should be more than a few centimetres from a particle of BHC. Nearly five tons of D. 919 were used to treat 312,500 square metres of river (fig. 1) between 26th January and 1st February 1955.

It is difficult to assess the results of such an experiment. The larvae are unevenly distributed on the bottom so numerous samples of them are required. It takes a long time to obtain these and still longer to separate the larvae from débris and examine each one. Accordingly, drifting pupae were collected in tow-nets moored in three positions (fig. 8). One was 20 metres south of the Palace buoy and 50 metres below the treated area, another above the treated area attached to a moveable buoy which was fixed in position on 29th January, and a third for a short time 350 metres below the treated area. Two half-hour catches were made nightly with each net, the first beginning at dusk, half an hour after sunset. It was estimated that a pupa reaching the surface about dusk at the upstream end of the treated area would have passed or entered the Palace net an hour after dusk, and that, if the larvicide were effective, the catches in the Palace net would probably diminish. They did not do so (fig. 8) but this did not necessarily imply that the larvicide was ineffective. It is conceivable that pupae rise very slowly and that those caught had come from above the treated area. Additional information was therefore sought by collecting larvae. Dr. J. Rzóśka took six samples on 22nd March between 1700 and 1800 hr. with a grab which covered 400 sq. cm. of the bottom and took up mud from a depth of 5 or 10 cm. The points sampled were 25, 300, 600, 750, 1,070 and 1,600 metres upstream of the centre of the Palace, the fifth being in mid-stream, and the numbers of larvae as follows:—

Sample	<i>Tanytarsus</i>	Other genera
1	12	6
2	7	15
3	8	358
4	95	66
5	226	55
6	89	57

Of the eight *Tanytarsus* larvae in sample 3, six had evidently died before capture. This and the relatively small number of *Tanytarsus* from the treated area suggest that the larvicide, long after application, was having some effect on *Tanytarsus*, but the normal distribution of these larvae is not known. The larvae of other genera, most of which had a pinkish tinge, may have been under the mud and so escaped being affected. No dead fish were seen after this, or any other, experiment.

At this stage, two insecticide formulations, designed to sink quickly and spread on reaching the bottom, were submitted, 5 per cent. γ BHC in kieselguhr by Messrs. Imperial Chemical Industries Ltd., and 5 per cent. DDT in bentonite by Messrs. Shell Company Ltd. Both materials expand considerably in water. A pellet of the latter soon becomes a cone with about 20 times its original base area and double its height, and tends to be spread further by the movements of aquatic insects. In preliminary laboratory tests these materials were treated like the granular BHC and commercial DDT, respectively, as described above. They had the same effect on larvae as the granular BHC, but field trials can only be considered after a study of costs.

An attempt to kill pupae.

Fifty per cent. DDT wettable powder was placed in a dish at the rate of 50 parts of DDT per million of water, stirred and left standing. Pupae were left in the dish for half an hour, often in contact with DDT, without apparent result.

Prevention of emergence by oiling.

This has not been found very effective in other countries but was tried at Khartoum owing to the difficulty of finding an alternative. Preliminary laboratory tests with a thin film of waste garage oil showed that the mesonotum of the pupa rose above the water level and the adult emerged successfully, only to be trapped by the oil when it stepped off the old pupal skin. The oil was available in large quantities free of charge and spread well on the clean surface of the river at the rate of about one litre to 5,000 sq. metres. Application from fixed points on the right bank and the bridge was unsatisfactory because wind blew the oil ashore before it covered the river, so oil was discharged from a launch cruising near the right bank between the dockyard and the bridge. Two four-gallon tins fitted to the stern had apertures giving a total flow of one gallon a minute. The wind and current carried the oil south-west and each run of the boat replenished it during the main period of emergence. Some 5,000 litres of oil were applied in this way between 12th and 23rd January 1953, usually from 0330 to 0600 hr. and sometimes longer. Many dead newly-emerged midges were found, particularly in patches of thick oil, but large numbers continued to appear at Khartoum, although an improvement was reported from Khartoum North. It was concluded that the film had been incomplete owing to the effect of varying wind and waves and the difficulty of working in the dark. A second boat would be necessary for the west of Khartoum, but it seems unlikely that the method would protect the town.

Early morning oiling along the right bank should be of some use to Khartoum North because oil in the lee of the high bank is not carried away rapidly by wind.

Oil was used against *N. brevivucca* near the dockyard in March between 1745 and 1830 hr. Many adults were seen trapped in the oil and good results were reported by local residents. The sides of sailing boats had to be cleaned after the oiling of the river but no complaints were received of other effects.

Insecticidal fog.

A solution of 0.25 per cent. pyrethrins in diesel oil was applied by "Tifa" and "Swingfog" machines to clouds of Chironomids near buildings. Many were

killed but much insecticide had to be used because the midges are most troublesome on windy evenings so the period of exposure was short. The effect of wind on the flies enables fogging operations to be planned a few hours ahead.

Residual sprays.

When adults of *T. lewisi* were placed in petri dishes containing wettable-powder deposits of 0.01 gm. γ BHC and 0.1 gm. DDT per sq. metre they were incapacitated within 20 minutes. Trees and shrubbery in 3.83 hectares of gardens along a river frontage of 236 metres were sprayed with 3,180 litres of 0.25 per cent. DDT emulsion. Several residents reported an improvement for a week, but the results did not appear to warrant the oft-repeated sprayings which would be necessary to maintain an active residue.

Light-traps.

Light-traps have been used against *Chaoborus* at Clear Lake, California, but their use on a large scale was evidently not entirely satisfactory because the pest was eventually controlled by large-scale larviciding. It has been suggested that midges should be intercepted by light-traps before they reach Khartoum, but this would be impossible because so many emerge in daylight. A small trap with fan and receiving-bag caught many midges at night but many nearby failed to enter it. It was often noticed that when midges are attracted to lights many others remain at faint sources of light near or far away, such as white clothing and dimly lit walls. They are probably inhibited in the way mentioned by Robinson (1951). General use of light-traps at Khartoum would be very expensive and probably only partly effective.

Removal of vegetation.

The cultivation of beans on the river bank near the dockyard was once prohibited, and this removal of daytime resting places was said to give some relief. If trees were cut down north of such buildings as the University College, which cannot be vacated, the midge nuisance in their vicinity would be greatly reduced. Privacy could be retained by the use of raised balconies, at first-floor level, like that on the north side of the Palace (Pl. V, fig. 1).

A barrier of vegetation.

The first row of gardens along the river serves as a partial barrier to midges and confines to the vicinity of the river many which would otherwise be blown inland. This barrier could be made more effective by planting a row of trees of dense habit, such as mango and lime (*Citrus aurantiifolia*), at the back of these gardens about 100 metres from the river. The lay-out could be adjusted so that the trees did not attract midges to existing houses. The work could be started by planting short experimental barriers of quickly growing trees like neem (*Melia azadirachta*), and observing the effect on houses down-wind. Such a barrier would also reduce locally the hot dusty winds of summer.

Removal of people inland.

Many people have already moved from the river front to the southern part of the town. Several of the vacated houses have become offices, some of which are not used in the evenings. Since complete control of midges may not be feasible and even a few of them can be annoying, the only certain way of avoiding the pest may be to move inland. The loss of amenity involved is much less than it might seem because most of the river-front houses do not overlook the Nile from which they are separated by shrubbery and a road.

Personal protection.

If mosquito wire is placed over a source of light a few midges will pass through 0.75 mm. mesh, some through 1 mm. mesh and many through 1.5 mm. mesh.

Fine wire of 1 mm. mesh round a house will keep out most midges, but in practice enough enter, when doors are opened for instance, to cause annoyance, and finer wire would keep out the much-needed evening breeze.

Some residents obtain partial relief by using dim lights or red lights which are unattractive to the midges. Others place strong tubular lights on the outside walls of their houses. These attract the midges away from people sitting about ten metres away who can see to read. The use of dark clothing reduces annoyance.

The closing of doors and windows to exclude midges is impracticable owing to the climate, but equipment for cleaning or conditioning the air would no doubt be useful during the evening infestation.

Other measures.

Smoke fires are sometimes lit at the Mogren public gardens, near the junction of the Blue and White Niles, but have little effect on the midge nuisance. Damage to paint on steamers could perhaps be prevented by altering the dockyard lighting system.

Summary.

This paper deals mainly with species of *Tanytarsus* (CHIRONOMIDAE) at Khartoum, particularly the very common *T. lewisi* Freeman. These midges are a serious pest there, usually between November and April, causing great annoyance by swarming in vast numbers around lights during the first few hours after sunset and thus interfering with work and pleasure in the riverain area. They are probably responsible for a considerable amount of asthma and other conditions due to allergy. Little information is available about the biology of CHIRONOMIDAE in Africa, so reference is made to the Palaearctic *T. manicus* Walker, to which *T. lewisi* is closely related. Studies of Chironomids from the health point of view elsewhere have little relation to the Khartoum problem, but work on *Chaoborus* (CULICIDAE) is of interest.

The conditions under which these midges occur at Khartoum and the methods of study are described.

At least 26 species of CHIRONOMIDAE, including four of *Tanytarsus*, occur at Khartoum. The respiratory organs of some pupae are figured, and a key, based on the characters of these organs, is given for some of the species.

Observations on the biology of all stages are recorded, particularly on the vertical movements and drifting of larvae and pupae and the time of emergence. Many larvae and pupae drift downstream at night, and *T. lewisi* emerges mainly in the early morning.

Some exploratory field trials of larvicides were carried out. The results were inconclusive, and the difficulty of assessing them is pointed out. Thorough control by anti-larval measures would be extremely difficult owing to the large size of the river and the drift of pupae from upstream. It is believed that it might be possible but prohibitively expensive, and would have to be repeated annually, perhaps throughout the midge season. Various protective measures are discussed. It is considered that riverside dwellers who can do so should move inland, and that a barrier of trees parallel to the river would protect houses away from the river front. People who must be near the river in the evening can achieve considerable protection by clearing some vegetation, fogging with insecticide; or using air-cleaning or air-conditioning equipment.

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FIG. 1. View of Khartoum from the north-east showing the left bank of the Blue Nile, the Palace, and trees which shelter the Chironomids.



FIG. 2. A tow-net in use at Wad Medani in April. The current here makes a boat unnecessary.



FIG. 3. A tow-net showing bottle used as float, metal tube with floats and detachable bag.



FIG. 4. View to the north from the Ministry of Works steps at Khartoum, showing a launch discharging larvicide into the Blue Nile.

A LIFE-HISTORY STUDY OF *ANTHRENUS FLAVIPES* LEC. (COL., DERMESTIDAE).

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Of the several species of Dermestid beetles whose economic importance is now widely recognised, *Anthrenus flavipes* Lec. (*vorax* Waterh.) may be regarded as the most widely distributed species found in India. The principal accounts of the biology of the species are those by Herfs (1933, 1936), Back & Cotton (1937), Griswold & Greenwald (1941) and Sohi (1951). Hinton (1945) has described in brief the results reported by all the above authors with the exception of the last. Sohi (1951) has not described the conditions under which he carried out his studies. Since, however, current knowledge was not adequate for the rearing of standard cultures of this species and for the establishment of a standard test procedure for the assessment of insect-proofing agents on woollen textiles, a systematic study of the life-history of the insect was undertaken in these laboratories. This forms the subject matter of the present communication. In the present study, hog bristles were used in preference to wool because larval development is quicker on this medium and the bristles facilitate the counting and removal of eggs and the recording of larval moults. A suitable test procedure has also been evolved using *A. flavipes* as the test insect but an account of this work will be published separately elsewhere.

Occurrence and Distribution.

A. flavipes was first reported to occur in India by Cotes (1890, p. 208) who found it on dried mammalian skins in the Indian Museum, Calcutta. According to Back (1931) this species was known as the Furniture Carpet Beetle in the United States and occurred in Algeria, Spain, Greece, southern Russia, Mesopotamia and the East Indies. It has also been recorded in the Sudan (Anon., 1918), Egypt (Willecocks, 1922), Sahara (Foley & de Peyerimhoff, 1922), Germany (Zacher, 1930) and China (Patton, 1931).

The adult beetles feed under normal conditions only on pollen and nectar (Hinton, 1945) and honey (Herfs, 1936). The larvae are generally found feeding on wool, hair, feathers, bristles, fur, horn and tortoise-shell (Hinton, 1945). Back & Cotton (1937), as reported by Hinton (1945), found that the larvae occasionally gnaw holes in cardboard wrappings and damage cotton, linen, rayon, silk, jute, leather, soft wood and other materials "if these are stained or impregnated with animal excretions or other suitable matter". They also found that the "larvae skeletonise dead mice and eat dead insects, cheese, old grain, casein, dried blood and the glue in book bindings". The larvae have been recorded as feeding on the exuviae in the nests of the moth, *Thaumetopoca pityocampa* (Schiff.) (Foley & de Peyerimhoff, 1922). Hinton (1945) quotes Herfs (1933) as recording that in extreme hunger, materials such as cotton fabrics, silk, some artificial silks, sponges and dry cheese are eaten. Herfs (1936) has stated that a growth factor, known as "vitamin B₃" at the time of his studies, is also necessary if the larvae are to complete their development.

Life-history.

Insects for investigations on the life-history were drawn from laboratory cultures which were themselves derived from an infestation of woollen textiles in

a local store-house. The insects were collected in the larval and pupal stages and freshly emerged adults were used in the present studies.

Life-history studies were conducted (1) in incubators at 25, 30 and 35°C. and at two different humidity levels, 30 and 90 per cent., and (2) in a room where the temperature fluctuated between 25 and 31°C. over the year, and where the humidity was controlled at 70-75 per cent. R.H. by a hair hygrometer activating an electronic switch.

In the controlled experiments, the requisite humidities were maintained by the use of suitable concentrations of potassium hydroxide in water (Buxton & Mellanby, 1934). The solutions of potassium hydroxide were introduced into desiccators which were then placed in incubators.

The experimental data have been summarised and tabulated as the mean (M), and standard error of the mean (SE), for the observations recorded.

TABLE I.

Incubation period and percentage viability of eggs.

Temp. (°C.)	R.H. (%)	Number of eggs examined	Incubation period (days)			Viability of eggs (%)		
			M	±	S.E.	M	±	S.E.
10-12	90	25	Failed to hatch*					
25	30	75	13.73	±	0.07	60	±	5.7
	90	70	13.63	±	0.08	66	±	5.7
30	30	95	8.64	±	0.06	77	±	4.3
	90	110	8.13	±	0.04	77	±	4.0
35	30	75	6.52	±	0.06	85	±	4.1
	90	85	6.69	±	0.06	78	±	3.3
25-31	70 - 75	146	6.81	±	0.07	87	±	2.8
37.5	40 - 50	30	4.46	±	0.10	87	±	6.1
40	40 - 50	30	Failed to hatch **					
41.5	Exposed for 30 minutes†	30	20	Failed to hatch **				
	Exposed for 15 minutes †	90	20	Failed to hatch **				

In view of the low S.E., maximum and minimum incubation periods have not been given. The standard error for percentage viability of eggs was calculated as the $M \pm S.E.$ of the mean of the total number of eggs examined.

* The eggs remain normal in appearance for 30 to 35 days and then shrivel up. Observations were discontinued after 45 days as by that period all the eggs had dried up.

** The eggs remain normal in appearance for 2 to 3 days and then shrivel up. Observations were discontinued after 10 days as by that period all the eggs had dried up.

† The eggs were subsequently kept in an incubator at 33°C. for observations on viability.

The egg.

The eggs of *A. flavipes* are elongate oval in shape, slightly broader at one end than at the other and twice as long as broad. The dimensions of 30 eggs were as follows:—

	Length (mm.)	Width (mm.)
Maximum	0.708	0.361
Minimum	0.542	0.278
Mean	0.643 ± 0.031	0.319 ± 0.030

The surface of the egg is somewhat wrinkled and at one end bears a number of spine-like projections which serve to attach it to the surface upon which it is deposited. The egg, when freshly laid, is iridescent white but gradually changes to a pale yellowish colour. Just before hatching, the head capsule and the body hairs of the larva become visible through the egg-shell.

Incubation period and percentage viability of eggs.—A known number of eggs (about 8 hours old) was incubated at different temperatures and humidities. These eggs were examined daily and the date of hatching of individual eggs was recorded. The results for incubation period and percentage viability are summarised in Table I. For purpose of comparison, the corresponding data of Herfs (1936) and Griswold & Greenwald (1941) as summarised by Hinton (1945) are shown in Table IA.

TABLE IA.

Incubation period of eggs (after Herfs; figures in brackets after Griswold & Greenwald).

Temp. (°C.)	R.H. (%)	Number of eggs	Days			
			Mode	Mean	Min.	Max.
(18.3)	—	(37)	—	(32.27 ± 0.13)	(31)	(34)
20.0	35-40	650	31	—	—	—
(23.8)	—	(83)	—	(17.01 ± 0.06)	(15)	(18)
25.0	35-40	767	16	—	—	—
(29.4)	—	(36)	—	(9.98 ± 0.08)	(9)	(11)
30.0	35-40	2835	10	—	6	13
35.0	35-40	1686	8	—	—	—
40.0	35-40	1000	all died	—	—	—

The duration of the egg stage was inversely related to temperature. Humidity had no effect on the length of the incubation period. This conclusion is in agreement with that of Herfs (1936). Fluctuations in temperature, between 25 and 31°C., were more favourable to the incubation period than constant temperatures of 25 and 30°C.

The percentage viability of eggs was higher at 25 to 31, 35 and 37.5°C. than at 25 and 30°C. The fluctuations in temperature between 25 and 31°C. were more favourable for the viability of eggs than the constant temperatures, 25 and 30°C. and about equal in this respect to 35°C. Eggs failed to hatch at 10 to 12°C. and 90 per cent. R.H. and at 40°C. and 40 to 50 per cent. R.H. when exposed to these conditions continuously but when exposed for 15 minutes at 40°C. and 30 and 90 per cent. R.H. they were viable. The eggs exposed for 15 minutes at 41.5°C. and 90 per cent. R.H. failed to hatch but at 41.5° and 30 per cent. R.H. for the same

exposure the viability was 20 per cent.; eggs at 41.5°C. and 30 per cent. R.H. exposed for 30 minutes failed to hatch.

At favourable temperatures, humidity did not appreciably affect the viability of eggs. This is in agreement with the finding of Herfs (1936).

The larva.

The newly hatched larva is creamy white, elongate oval and densely clothed with dark hair. The dimensions of 20 larvae on emergence were as follows:—

	Length (mm.)	Width (mm.)
Maximum	0.625	0.333
Minimum	0.528	0.278
Mean	0.591 ± 0.006	0.299 ± 0.004

The larvae moult a variable number of times before they reach maturity, and pupate in the last larval skin.

Larval period and number of instars.—The larval period and number and lengths of instars were studied at different temperatures and humidities by liberating larvae individually (about 8 hours old) in specimen tubes (1" × $\frac{1}{2}$ "), containing fragments of hog bristles. The results are shown in Table II.

TABLE II.

Number of larval instars and duration of larval period.

Temp. (°C.)	R.H. (%)	No. of larvae that completed development	No. of instars			Total larval period (days)	
			Min.	Max.	Mean	M ±	S.E.
30	30	41	14	21	18.68	327.9 ±	4.60
	90	31	15	20	18.96	333.5 ±	5.15
35	30	8	18	21	18.87	221.5 ±	5.62
	90	5	18	20	19.0	221.4 ±	5.15
25-31	70-75	40	14	19	15.8	222.6 ±	4.51

The higher the temperature the shorter the larval period. A temperature fluctuating between 25 and 31°C. was more favourable to rapid larval development than a constant temperature of 30°C. and about equal to 35°C.

According to Herfs (1936) there is no appreciable difference in the larval period at 30 and 35°C., but it is much shorter at these temperatures than at 20 and 25°C. His larvae developed over twice as quickly as those in the present work, no doubt because the food material (wool impregnated with an extract of horse-dung) was more suitable. Herfs found a retardation of the rate of development at fluctuating as compared with constant temperature unlike the marked acceleration recorded above. This may have been because the upper temperatures he used were unfavourably high (40°C.).

Humidity had no effect on the duration of the larval period at the temperatures investigated. According to Herfs (1936) the larval period is shorter at 90 to 100 per cent. R.H. than at 30 to 40 per cent. R.H.

The number of larval instars, as determined by the number of moults, and duration of each instar were noted, and the summarised results are included in Table II.

The number of larval instars at 30 and 35°C. was the same. The fluctuating temperature (25 to 31°C.), however, reduced the number of instars although the total larval period was the same at this temperature as at 35°C. The duration of each instar was less therefore at 35°C. than at the fluctuating 25 to 31°C. Herfs (1936) has recorded that the number of instars of larvae fed on wool +100 per cent. ZT* was more at 35°C. than at 30°C. and 30 to 40 per cent. R.H. According to him the duration of each instar is less at 35°C. than at 30°C. and 30 to 40 per cent. R.H.

Humidity had no influence on the number of larval instars and on the duration of each instar at the temperature levels investigated. Herfs' work, however, shows that high humidity reduces the number of larval instars.

Incidence of mortality during larval development.—The incidence of larval mortality was investigated at different temperatures and humidities by

TABLE III.

Mortality amongst larvae during development under different conditions of temperature and humidity. (Herfs' figures in *italics*.)

Temp. (°C.)	R.H. (%)	No. of larvae examined	Mortality (%)
20	35 to 50	31	19.3
25	35 to 50	20	5.0
25 to 31	70 to 75	84	51.8†
30	{ 35 to 50	20	0.0
	{ 30	97	57.7
	{ 90	62	77.4
35	{ 35 to 50	20	5.0
	{ 30	96	91.6
	{ 90	47	83.0
30 to 40	35 to 50	20	10.0
35 to 40	35 to 50	20	10.5
20 to 40	35 to 50	20	100
40	35 to 50	72	100
42.5	{ 30	10	100*
	{ 90	10	100*

* Did not survive exposure for one hour.

† For mortalities on other foodstuffs under these conditions, see Table IV.

liberating larvae (about 8 hours old), in specimen tubes (1" × ½") containing fragments of hog bristles. The number of larvae that died before completing the total larval period was recorded. The results are shown in Table III. For purposes of comparison, the corresponding data obtained by Herfs as summarised by Hinton (1945) are also shown.

* 100 per cent. ZT equals horse-dung boiled for a quarter of an hour with an equal weight of water and afterwards pressed through a fine mesh. The smooth, undyed wool (Papillonstoff) is evenly soaked with this mixture. The mixture is described by Titschack (1926, p. 511).

The incidence of mortality of larvae was lower at 30°C. and 25 to 31°C. than at 35°C. Larvae did not survive an hour's exposure at 42.5°C. irrespective of whether the relative humidity was 30 or 90 per cent. Herfs (Table III) also found that at 40°C. the larvae do not survive, but he records 90 per cent. survival where the temperature fluctuated between 30 and 40°C., though with a wider fluctuation of from 20 to 40°C. he found no survival. There is, however, wide difference from the present results at other temperatures. In particular, the results at 30 and 35°C. are not in agreement with the much lower mortalities recorded by Herfs. This is in keeping with the much faster development and fewer instars that he records.

TABLE IV.

Effect of different food materials on the larval development.

Description of food material	Nutrient (either in suspension or solution)	Concentration of nutrient in immersion baths (%)	Larval period (days)			Survival of larvae (%)
			Mean	Min.	Max.	
Hog bristles	—	—	223	158	283	46
Woollen fabric	—	—	339	287	406	28
	Cow-dung extract	—	218	194	241	98
	Glucose and albumin	20 of each	253	196	326	60
	Yeast	20	38	30	46	100
		15	41	30	49	100
		10	40	30	49	95
		5	118	100	140	95
		1	162	123	206	73
	'Centrifuged' yeast	20	65	28	102	100
		10	84	40	125	100
Fabric made of cotton and wool (50 : 50)	—	—	340	267	406	32
Cotton fabric	—	—	Larvae failed to develop			
	Yeast	10	"			
Silk fabric	—	—	"			
	Yeast	10	"			

Effect of different food materials on the development of larvae.—The development of larvae was investigated on wool, cotton, silk, cotton/wool (50:50 mixture) fabrics and on hog bristles. The wool, cotton and silk fabrics were impregnated with different materials (see Table IV) by immersing them in a bath

of the appropriate suspensions or solutions for 15 minutes. The glucose-albumin solution contained 20 per cent. glucose and 20 per cent. albumin in water. The aqueous extract of cow-dung used for impregnation was prepared by mixing fresh cow-dung with an equal weight of water and filtering the suspension.* The hog bristles were untreated. Twenty five to forty larvae (about 8 hours old) were placed on pieces of fabric, 2" \times 1", or fragments of bristles. The duration of the larval period and percentage survival of larvae were studied at 25 to 31°C. and 70 to 75 per cent. R.H. The results are summarised in Table IV.

The development of larvae was quickest on woollen fabrics treated with 10 to 20 per cent. yeast. The larvae did not develop on silk and cotton fabrics even when impregnated with yeast at 10 per cent. The development periods of larvae on untreated woollen fabric and cotton/wool fabric (50:50 mixture) were nearly the same and were prolonged. Treatment of the fabric with yeast, cow-dung and glucose-albumin favoured the development of larvae. According to Herfs (see Hinton, 1945, p. 340), larvae did not develop on pure wool whereas there was 100 per cent. development when pure wool was impregnated with 100 per cent. horse-dung extract (ZT). He has also shown that the larvae did not pupate on wool treated with horse-dung extract in concentrations of 50 per cent. or less. The results obtained in the present investigations have shown that about 30 per cent. of larvae successfully developed on untreated woollen fabrics although the larval period was considerably prolonged.

The pupa.

The skin of the full-grown larva splits on its dorsal side and the pupa remains enclosed in it during its entire pupal stage. The pupa is creamy white in colour. The dimensions of 20 pupae were as follows:—

	Length (mm.)	Width (mm.)
Maximum	17.8	10.0
Minimum	9.4	7.0
Mean	11.7 \pm 1.3	8.4 \pm 0.5

Sex-differentiating characters.—The sex-differentiating characters of the male and female pupae were investigated. In the female pupa, the eighth abdominal segment possesses characteristic finger-like lobes, the gonapophyses. Lobes

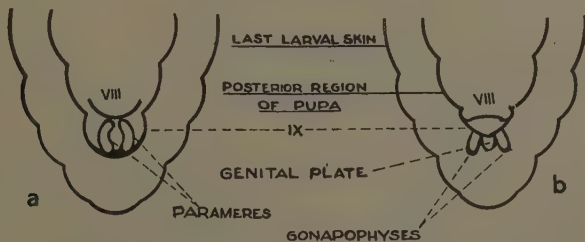


Fig. 1.—Male and female pupae of *A. flavipes*. a, male pupa with parameres not finger-like; b, female pupa with finger-like gonapophyses.

(parameres), although present in the male pupa, do not form the characteristic finger-like structures seen in the female (fig. 1).

Pupal period.—The duration of the pupal stage was studied at different temperatures and humidities by placing the pupae (about 8 hours old) between

* According to Lillie, Denton & Bird (1948) the growth factor in cow-dung extract is probably vitamin B₁₂.

two layers of pieces of woollen blanket. The weight per square yard of the blanket was $18\frac{1}{4}$ oz. The results are summarised in Table V. For purposes of comparison, the corresponding data of Herfs, summarised by Hinton (1945), are included in the Table.

TABLE V.

Length of pupal period, and percentage emergence of adults under different conditions of temperature and humidity. (Herfs' figures in *italics*.)

Temp. (°C.)	R.H. (%)	No. of pupae examined		Pupal period (days)						Emergence of adults (%)	
		♂♂	♀♀	M	±	S.E.	M	±	S.E.	♂♂	♀♀
25	30	27	20	13.95	±	0.21	13.67	±	0.20	55.6	60.0
	35-40	20		12.9			13.4			100	
	90	10	10	13.29	±	0.15	13.40	±	0.14	50.0	60.0
25-31	70-75	50	35	12.34	±	0.19	11.76	±	0.13	68.0	68.5
30	30	13	10	8.91	±	0.31	8.86	±	0.39	69.2	70.0
	35-40	20		9.0			10.1			100	
	90	10	18	8.50	±	0.19	9.43	±	0.37	60.0	72.2
35	30	20	20	7.53	±	0.11	7.31	±	0.18	75.0	70.0
	35-40	20		6.5			6.5			100	
	90	15	20	6.75	±	0.10	7.07	±	0.22	73.3	70.0
30-40	35-40	20		6.8			6.6			100	
35-40	35-40	20		7.9			7.4			100	
40	40	72		—			—			0	
40	50-60	?		—			—			16-18	
42.5	30	—	20	Did not survive exposure for 2 hours							
	90	—	20	Did not survive exposure for 1 hour							

The standard error was calculated as the S.E. of the mean of total number of pupae examined.

It should be noted that, whereas with incubation of eggs and development of larvae the fluctuating temperature (25-31°C.) is more favourable than a constant temperature of either 30 or 35°C., this is not so with the pupae. Temperature influenced the duration of the pupal stage, which was shorter at 30 and 35°C. than at 25 and 25 to 31°C. It was nearly the same for the male as for the female. These observations are in agreement with those of Herfs who has reported that the duration of the pupal stage of both the male and female is inversely related to temperature and that there is no appreciable difference in the duration of the pupal stage of either sex. Herfs' figures correspond fairly well with those in the present work. According to Griswold & Greenwald (1941), however, the pupal period in the males is slightly shorter than in the females. Humidity had no influence on the pupal period.

At higher temperatures (30 and 35°C.) the percentage emergence of adults was greater than at 25°C., but never exceeded 75 per cent. Herfs, however, obtained 100 per cent. emergence at all temperatures below 40°C. Humidity, however, did not appreciably influence the percentage of pupae emerging as adults at the temperatures investigated.

The pre-emergence (quiescent) period.—The fully formed adult casts off the pupal skin and remains within the last larval skin for a certain period which may be termed the pre-emergence or quiescent period. Later, it emerges from the last larval skin and becomes active. The duration of the pre-emergence period

was investigated at different temperatures and humidities. The results for the two sexes are summarised in Table VI. Figures obtained by Herfs (see Hinton, 1945) at corresponding temperatures, but at a single relative humidity, are included in Table VI.

TABLE VI.

Pre-emergence period of adults. (Herfs' figures in *italics*.)

Temp. (°C.)	R.H. (%)	No. of pupae examined		Pre-emergence period (days)					
		♂♂	♀♀	M	♂♂ ±	S.E.	M	♀♀ ±	S.E.
25	30	27	20	7.33	±	0.28	8.17	±	0.08
	<i>35-40</i>	20		<i>7.5</i>			<i>5.2</i>		
	90	10	10	8.40	±	0.21	8.33	±	0.17
25-31	70-75	50	35	7.41	±	0.34	8.12	±	0.60
30	30	13	10	7.67	±	0.14	6.57	±	0.17
	<i>35-40</i>	20		<i>4.7</i>			<i>3.7</i>		
	90	10	18	6.17	±	0.12	6.08	±	0.30
35	30	20	20	5.47	±	0.11	6.07	±	0.23
	<i>35-40</i>	20		<i>3.3</i>			<i>2.8</i>		
	90	15	20	4.82	±	0.25	4.86	±	0.32
<i>30-40</i>	<i>35-40</i>	<i>20</i>		<i>2.4</i>			<i>3.5</i>		
<i>35-40</i>	<i>35-40</i>	<i>20</i>		<i>2.9</i>			<i>4.4</i>		

While temperature bore an inverse relationship to the pre-emergence period, humidity had no appreciable influence. Sex also had no effect on the duration of the pre-emergence period. Herfs found that the pre-emergence period is influenced by temperature and sex. Griswold & Greenwald (1941) also have recorded that the pre-emergence period is influenced by temperature and sex.

The adult.

Sex-differentiating characters.—Griswold & Greenwald (1941) and Hinton (1945) have reported that the adult female is externally identical with the male. A sex-differentiating character has been determined in these studies. In



Fig. 2.—Ventral view of the last three segments of male and female adults of *A. flavipes*. a, in the male beetle the last segment bears a dark triangular patch; b, in the female beetle the last segment bears a dark trapezoidal patch.

the adult male, the ventral side of the last abdominal segment has a triangular dark brown patch, whereas in the female the patch, although dark brown, is trapezoidal in shape (fig. 2).

Length of life.—At the end of the quiescent period the adult escapes from the last larval skin and immediately starts moving actively. Under natural conditions the adults have been reported (Hinton, 1945) to feed on pollen and nectar; in the laboratory they have been successfully maintained on glucose-albumin solution and also on extract of cow-dung prepared as described earlier in this paper. Fragments of bristles provided in the oviposition tubes during the course of this investigation were smeared with these materials. The length of life of the adult, male and female, on these food materials was studied at different temperatures and humidities. The results are summarised in Table VII.

TABLE VII.

Length of life of adults.

Temp. (°C.)	R.H. (%)	Food material	Number of pairs	Length of life (days)		
				M ♂ ± S.E.	M ♀ ± S.E.	
25	50 to 60	Hog bristles smeared with glucose-albumin	7	36.3 ± 0.8	40.6 ± 2.7	
30	30	"	12	16.4 ± 0.4	17.4 ± 0.9	
	90	"	12	16.1 ± 1.8	16.3 ± 1.0	
35	30	"	12	14.7 ± 1.1	14.3 ± 0.6	
	90	"	17	18.1 ± 1.2	16.4 ± 0.8	
25 to 31	70 to 75	Hog bristles smeared with glucose-albumin	12	23.3 ± 1.6	25.5 ± 1.9	
		Hog bristles smeared with cow-dung extract	19	18.6 ± 1.2	23.0 ± 1.3	
		Hog bristles only	11	18.1 ± 1.8	26.3 ± 1.7	

Temperature had a marked effect on the duration of the adult stage. The adults lived longer at 25°C. than at 25 to 31, 30 and 35°C. There is no appreciable difference between the length of life of the adults at 30 and 35°C. At 25 and 25 to 31°C. the female lived longer than the male, but at 30 and 35°C. the male and the female lived for about the same period.

Humidity had no appreciable effect on the length of life of adults at 30°C. At 35°C., however, its duration in both males and females was greater at 90 than at 30 per cent. R.H.

The nature of food influences the length of life of both males and females. At 25 to 31°C. the adults lived longer on hog bristles smeared with glucose-albumin than on hog bristles smeared with cow-dung extract. There was, however, no difference in the length of life of females when released on hog bristles alone, or on hog bristles smeared with glucose-albumin, but the males lived longer on the latter. Herfs (1936) has stated that the length of life of the adult is influenced by sex, temperature, humidity and probably by the amount of food available and that the duration of life of the active adult depends to some extent on whether or not it has mated.

Pre-oviposition, oviposition and post-oviposition periods and number of eggs laid by a female.—Pairs of adults (about 8 hours old) were released in glass

dishes ($1\frac{1}{2}$ " \times $\frac{1}{2}$ "") containing fragments of hog bristles smeared with 20 per cent. glucose-albumin solution in water or an aqueous extract of cow-dung. The bristles, smeared with these materials, were replaced daily during the course of the investigation. The pre-oviposition, oviposition and post-oviposition periods and the number of eggs laid by a female were investigated at different temperatures and humidities. The results are summarised in Table VIII.

TABLE VIII.

Pre-oviposition, oviposition and post-oviposition periods and number of eggs laid by a single female.

Temp. (°C.)	R.H. (%)	Food material for adults	Number of pairs studied	Pre- oviposition period (days)	Oviposition period (days)	Post- oviposition period (days)	Number of eggs laid per female
				M \pm S.E.	M \pm S.E.	M \pm S.E.	
25	50 to 60	Hog bristles smeared with glucose- albumin	7	7.8 \pm 2.5	12.0 \pm 3.3	20.4 \pm 4.0	26 \pm 4.0
30	30	"	12	5.2 \pm 1.0	4.0 \pm 0.9	8.5 \pm 1.5	27 \pm 5.0
	90	"	12	6.3 \pm 1.3	5.3 \pm 1.1	5.0 \pm 0.5	21 \pm 4.0
35	30	"	12	5.8 \pm 0.8	3.1 \pm 0.6	5.9 \pm 0.6	19 \pm 2.4
	90	"	17	6.4 \pm 0.8	5.1 \pm 0.7	5.0 \pm 0.6	21 \pm 1.9
25 to 31	70 to 75	Hog bristles smeared with glucose- albumin	12	6.0 \pm 0.3	10.0 \pm 1.7	8.8 \pm 1.5	36 \pm 5.3
		Hog bristles smeared with cow-dung extract	19	8.3 \pm 0.8	7.6 \pm 0.9	8.5 \pm 1.3	32 \pm 3.0
		Hog bristles only	11	7.0 \pm 0.8	6.0 \pm 1.0	14.0 \pm 1.3	13 \pm 0.6

The pre-oviposition, oviposition and post-oviposition periods at 25°C. and 50 to 60 per cent. R.H. were longer than at 30 and 35°C. and 30 and 90 per cent. R.H. Lower humidity (30% R.H.) tended to reduce the oviposition period. It was observed that the females released on bristles smeared with glucose-albumin or cow-dung extract laid a larger number of eggs than those released on plain bristles. Temperature influenced the number of eggs laid by the female. The fluctuating temperature was more favourable than the other temperatures investigated.

Sex ratio.—Investigations on sex ratio were carried out on a number of cultures raised in the laboratory at 25 to 31°C. and 70 to 75 per cent. R.H. Out of 368 adults examined on emergence it was found that 45 per cent. were males and 55 per cent. were females, a ratio of 0.8:1. Herfs (1936) has recorded that the sex ratio is 48.1:51.9.

Duration of life-span.

The duration of the life-span (egg to death of adult) of the insect, reared on the diet described, was estimated on the basis of data given earlier for the various stages of the insect. Estimates at different temperatures and humidities are shown in Table IX.

TABLE IX.

Life-span of *A. flavipes*.

Temp. (°C.)	R.H. (%)	Life-span in days	
		Male	Female
30	30	370	370
	90	370	370
35	30	243	252
	90	248	246
25 to 31	70 to 75	273	277

The duration of the entire life-span of the insect, from the egg to the death of the adult, was inversely related to temperature. The overall span was, however, the same for both male and female at any temperature-humidity level. Thus, under the conditions described in these experiments, there are one to one-and-a-half generations of the insect in a year depending upon temperature conditions.

Natural Enemies.

Back (1940) has recorded that the larva of a Bethyloid, *Laelius voracis* Mues., sometimes completely destroys cultures of *A. flavipes*. Ayyappa & Cheema (1952) have described an ectoparasite (on the larva of *A. flavipes*) considered to be an undescribed species of *Laelius* and have worked out its life-history in detail.

Summary.

Anthrenus flavipes Lec. (*vorax* Waterh.), commonly known as the Furniture Carpet Beetle, is a widely occurring species of wool-destroying insect in India. It feeds on woollen materials, feathers, bristles, furs, horse-hair, horny substances and other materials of a keratinous nature. The adult beetles feed on pollen and nectar of flowers in nature and are harmless; and it is the larva which destroys the above materials. The life-history of the pest has been studied at several controlled levels of temperature and humidity, and also in a room where the humidity was controlled at 70–75 per cent. R.H. and the temperature fluctuated between 25 and 31°C. over the year. The results are compared with those of earlier authors. The sex-differentiating characters have also been investigated in the pupa and adult.

Temperature influences the duration of the egg stage and the viability of eggs. The incubation period of eggs at 25, 30, 25 to 31, 35 and 37.5°C. is about 13, 8, 7, 6 and 4 days, respectively. The viability of eggs is greater at 25 to 31, 35 and 37.5°C. than at 25 and 30°C. The fluctuating temperature (25 to 31°C.) has a more favourable effect on the incubation period and viability of eggs than the constant temperatures of 25 and 30°C. Humidity, however, has no effect on either the duration of the egg stage or the viability of eggs.

Temperature has a marked effect on the total larval period and to a lesser extent on the number of larval instars. The larval period is appreciably less at 25 to 31°C. and 35°C. than at 30°C. The number of larval instars is greater at 30 and 35°C. than at 25 to 31°C. though the duration of each instar is less at 35°C. than at 25 to 31 and 30°C. The fluctuating temperature (25 to 31°C.) is more favourable to rapid larval development than the constant temperature of 30°C. and also reduces the number of instars. Humidity has no effect on the number of larval instars and the total larval period.

Larvae thrive most satisfactorily on woollen fabric treated with 10 to 20 per cent. commercial yeast.

The duration of the pupal period and the number of adults emerging are dependent on temperature. The pupal period is about 14, 12, 9 and 7 days at 25, 25 to 31, 30 and 35°C. and is nearly the same for the male and the female. Humidity has no effect on the pupal period. These results are discussed in relation to those of earlier workers.

The pre-emergence (quiescent) period extends over 7 to 9, 7 to 8, 6 to 8 and 5 to 6 days at 25, 25 to 31, 30 and 35°C., respectively, irrespective of sex. Humidity has no appreciable effect on this period. These results are at variance with those reported by earlier workers.

The length of life of the adults is dependent on temperature but humidity has no appreciable effect on this period. The nature of food influences the length of life of the adults. A female starts laying eggs 4 to 10 days after emergence, depending upon temperature and humidity, and lays 12 to 41 eggs. The sex ratio between male and female was found to be 0.8:1.

The entire life-cycle is shortest at 35°C. Under conditions similar to those of the experiments described, there are one to one-and-a-half generations of the insect in a year depending upon the temperature conditions.

Acknowledgements.

The authors desire to record their thanks to Dr. T. S. Subramanian and Mr. S. K. Ranganathan of these laboratories for their interest in the work. Their thanks are also due to the authorities of the British Museum (Natural History), London, for the identification of the insect species. The paper is published with the permission of the Director of Research & Development (General), Ministry of Defence (CGDP), New Delhi.

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ON THE PARASITES AND PREDATORS OF THE COCKROACH.

R II.—EVANIA APPENDIGASTER (L.).

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The first paper (Cameron, 1955) in this series introduced the subject of the biological control of cockroaches with a general account of the 70 or more natural enemies of these pests. It also contained a detailed study of one of the more important parasites, the Eulophid—*Tetrastichus hagenowii* (Ratz.). From an economic point of view some of the 70, particularly the Protozoans and the Nematodes, were shown to be relatively unimportant, but at least 30 or more of the Hymenopterous parasites and predators were considered to be worthy of further investigation. Amongst these the Evaniid parasite, *Evania appendigaster* (L.), appeared to be the next most important subject for further research. This species can be regarded in many ways as more or less typical of a group of ten ~~plus~~ ⁸⁷ members of the EVANIIDAE, all of which parasitise the egg-capsules of the commoner species of cockroach. The family EVANIIDAE (Ensign-flies) is a comparatively little-known section of the Hymenoptera-Parasitica which, in the words of one systematist (Crosskey) has been "sadly neglected by monographers". So far as the taxonomy of the group is concerned this neglect has been partially repaired by Townes (1949), who reclassified the Nearctic species, and by Crosskey (1951), who rearranged the British members of the family. Neither of these authors, however, contributed any new information about the biology and development of Evaniids, so the present position in this respect is again well and truly expressed by Crosskey in his taxonomic study of the group by the following statement—"With regard to the habits and life-histories of the British Evaniioidea the paucity of information is even more marked than in the taxonomy". Indeed, apart from an early paper by Genieys (1924) on the allied genus *Zeuxevania*, another by Edmunds (1952) on *Prosevania* and one or two brief notes, hardly anything has been published about the biology of the Evaniids proper. The present paper, therefore, while continuing the investigation along biological-control lines, should also serve as a new source of information about the biology and development of this neglected group. It deals mainly with the host records, distribution, biology and development of *E. appendigaster* together with some notes on the habits and distribution of several allied species. 87

General Biology of *Evania appendigaster*.*Collections and parasitism.*

The material (oöthecae of *Periplaneta americana* (L.)) on which this investigation was based came mainly from Jedda in Saudi Arabia. Collections of egg-capsules of *P. americana* were made in this area at different seasons of the year by my friend Dr. A. Zahar to whom I am much indebted for this service. The oöthecae were dispatched by air mail so that the time occupied in transit was reduced to a minimum. On arrival they were carefully examined for larvae and pupae of any parasites that might be present. The parasitism by *E. appendigaster* in this material averaged 25–29 per cent.

Generations.

So far as one can judge there are at least three, and possibly four generations of the parasite in the course of a year. This finding is based on the following

observations. A small collection of oöthecae, dispatched in January, was found on examination to contain a number of advanced larvae, prepupae and pupae of *Evania*. Although this particular batch of material was held up for several days, some adults emerged in the early part of February, and at least one of the females from this collection was observed ovipositing in a laboratory-bred egg-capsule of *P. americana*. This would suggest that the first generation of adults is on the wing in or about February. Much larger collections were received in March when the parasitism rate by *Evania* was 29 per cent., and again in October when it was 25 per cent. In March, most of the parasites were in the late larval stages, and adults from this collection kept emerging throughout the month of May, suggesting a second generation in the field during this month. In the October collection, *Evania* was found to be present mostly in the pupal stage, almost ready for emergence. This would suggest a third generation in or about October. Whether there is a fourth generation or not in the hot climate of Saudi Arabia is uncertain, but the above findings would seem to indicate that there are at least three generations, the adults of which emerge about February, in May and in October, respectively. In the allied genus *Prosevania*, Edmunds (1952) found that the species *punctata* (Brullé), a native of the Mediterranean region but introduced into North America, has three generations a year, one at the end of May, a second at the end of July, and a third in September–October. The average developmental period from oviposition to emergence in this latter species varied from 45 to 177 days and averaged 50 to 60 days. In Spain, Cros (1942), working on the same species as Edmunds, found that there were two, and possibly three, generations a year in that country, the developmental period in September–October being six to eight weeks. The first generation of adults appeared in June–July from eggs laid in the preceding year, while adults were common in houses in July, August and September. All these observations would indicate that *Evania* and its relatives, and possibly all the Evaniids which parasitise cockroach oöthecae, are very much more rapid breeders than their hosts. With three or more generations a year against one or less for the host, their usefulness as controlling agents should be correspondingly greater.

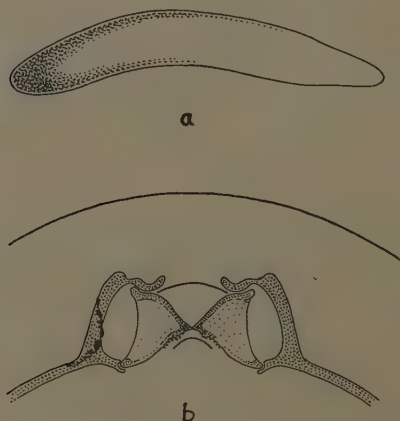


Fig. 1. *Evania appendigaster*: a, egg ($\times 41$); b, cephalic skeleton and mandibles of first-stage larva ($\times 98$).

Length of life.

The adult parasites live for only a comparatively short time. Those from the Saudi Arabia collections lived in captivity, in cages well supplied with food and water, for periods of up to two or three weeks.

Oviposition.

A number of oöthecae of *P. americana* taken from a laboratory culture of this species were stuck on to small pieces of sheet cork and introduced into cages containing adults of *E. appendigaster*. The insects soon became aware of the oöthecae and the females began to palpate them with their antennae. Before very long one female was seen to take up a rather unusual position in preparation for egg-laying. Instead of attacking the capsule from above she lay on her side with her body parallel to the long axis of the oötheca and legs braced against the latter and the cork in its vicinity. After a good deal of hard work, and much wriggling of the abdomen, she eventually managed to penetrate the tough integument of the egg-capsule and insert her ovipositor. The whole operation occupied about half-an-hour. Similar behaviour was observed in *Proscvania punctata* by Edmunds, when the time of penetration and subsequent waiting was 20–30 minutes. Genieys has suggested that, in the related genus *Zeuxevania*, oviposition may be effected while the oötheca is still soft and attached to the abdomen of the host. Such a method has not been observed in *E. appendigaster*, and its occurrence, in view of the method just described, is extremely unlikely.

Habit.

This parasite, unlike *T. hagenowii*, is solitary in habit during the developmental period. Only one adult emerges from a parasitised oötheca and all the host eggs are eventually devoured by the single developing larva.

Larval instars.

The number of larval instars was determined by examining the contents of oöthecae from which adult Evaniids had emerged. After treatment with a weak solution of KOH this residual material was dissected and the larval skins of the

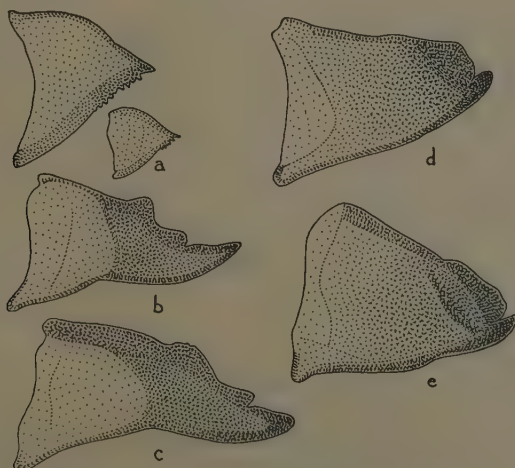


Fig. 2.—*Evania appendigaster*: a–e, mandibles of successive larval instars [a, $\times 210$ and 105; b–e, $\times 158$].

parasite, particularly the mandibles of the different stages, were sorted out. Identification of the separate instars was greatly facilitated by the distinctive shapes of the various mandibles. These are described and figured (fig. 2) in the following section. Five different sets of mandibles were dissected out from each of several parasitised oöthecae, thus indicating that there are five larval instars in this particular parasite.

Prepupal and pupal instars.

On completion of feeding, the parasite passes through a distinct prepupal stage. This is followed by a pupa of the exarate type. A cocoon is not formed.

Development of *Evania appendigaster*.

The egg (fig. 1, a) of this species is white in colour, the surface smooth and glistening. It measures about 1.3 mm. in length by 0.2 mm. in maximum breadth. In shape it is markedly arched with one end slightly more pointed than the other, in short this is a typical "hymenopteriform" egg.

The first-instar larva and the final one are described and figured here from direct observations on the larvae themselves but the data on the intervening instars is based on an examination of cast larval skins recovered from oöthecae from which *Evania* had emerged. The most striking features of the first-instar larva, as indeed of all the stages, are the mandibles. These are triangular in shape (figs. 1, b & 2, a), sharply pointed and particularly noteworthy because of the serried arrangement of small "teeth" which are present behind the main denticle. This unusual armature is probably useful to the larva when cutting through the tough shell of the host egg. The cephalic skeleton of this instar follows the usual plan of hypostoma, pleurostoma (rather broad), pleurostomal rami for articulation with the mandibles, and epistoma. The latter is incomplete medially.

The mature larva (fig. 3, a, b) is a greyish-white, fat and flabby grub. It consists of a head and 13 body segments. Ten of these are well marked, the next two smaller and retracted, the last short and indistinct, while the first partially envelops the retracted head. The glistening skin is devoid of any particular markings or excrescences apart from a few "sensillae" in the sub-oral region of the head. When contracted, as it often is, this final-instar larva has a very wrinkled appearance. The head (fig. 4) is somewhat spherical in shape with the sub-oral region particularly prominent. A pair of short broad-based antennae are present well above the mouth opening, and the cephalic skeleton with a pair of heavily chitinated bidentate mandibles shows up prominently (figs. 4 & 5) above the labium and maxillae. The epistoma is again not quite complete medially. Both the maxillae and the labium are adorned with "sensillae" and a few small papillae may be present on the labral area. Nine pairs of open spiracles (fig. 3, d) leading into well-developed tracheal trunks (fig. 3, b) are present on segments 2 and 4-11. Each spiracle has an ovoid atrium (0.09 mm. by 0.05 mm.) attached to a long reticulately sculptured tube, which in turn is joined to the short spiracular trachea.

The average measurements of the mature larva are 7-8 mm. in length by 3.5-4 mm. in breadth.

As the mandibles (fig. 2) of the various instars appear to have some diagnostic and taxonomic value they will be described here together. Those of the first instar (fig. 2, a), as already indicated, are triangular in shape and are easily recognised by the series of small spine-like teeth behind the main denticle. In the second and third instars this shape is partially lost and the mandibles of these two stages bear a curious resemblance to a gauntlet glove (figs. 2, b, c). They are also strongly and markedly toothed. Both may be described as

tridentate, possessing one large curved terminal denticle in the ventral position, and two blunter teeth more dorsally placed. The mandibles of the penultimate and final instars (figs. 2, d, e) also resemble each other, but without the gauntlet shape. They are now shorter and wider, and the clear-cut tridentate tip has been replaced by a narrow curved ventral denticle and a long blunter dorsal one which shows slight traces of a double structure. All the mandibles, with the exception of the first pair, are heavily chitinated for some distance behind and including the denticles.

The prepupa (fig. 3, c) which is white in colour, strongly resembles the mature larva except for the well-marked constrictions between the future head, thorax and abdomen of the imago superimposed on the old larval segmentation.

The pupa, like the prepupa, is at first white in colour but as development proceeds a gradual darkening sets in, first in the thoracic region, and later in the anterior part of the abdomen, until eventually the whole pupa is black and shiny.

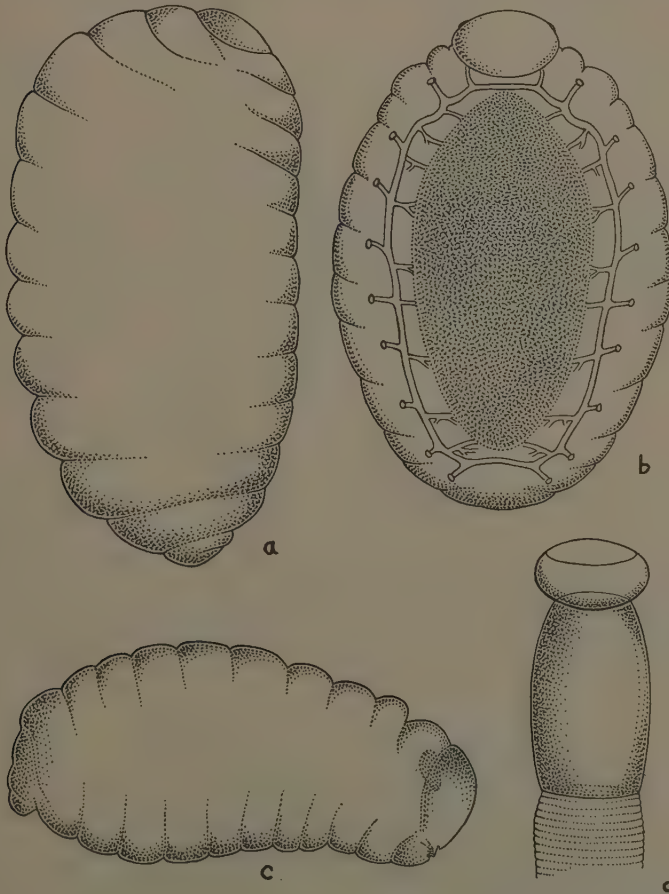


Fig. 3.—*Evania appendigaster*: a, mature larva ($\times 12$); b, mature larva showing tracheal system ($\times 12$); c, prepupa ($\times 10$); d, spiracle ($\times 200$).

At maturity the imago makes its escape through a small round jagged hole which it cuts near the end of one of the long sides of the oötheca.

A brief summary of recognition characters for the eggs, larvae and adults of *Evania appendigaster* is given below.

Eggs. Arched hymenopteriform type about 1.3 mm. in length.

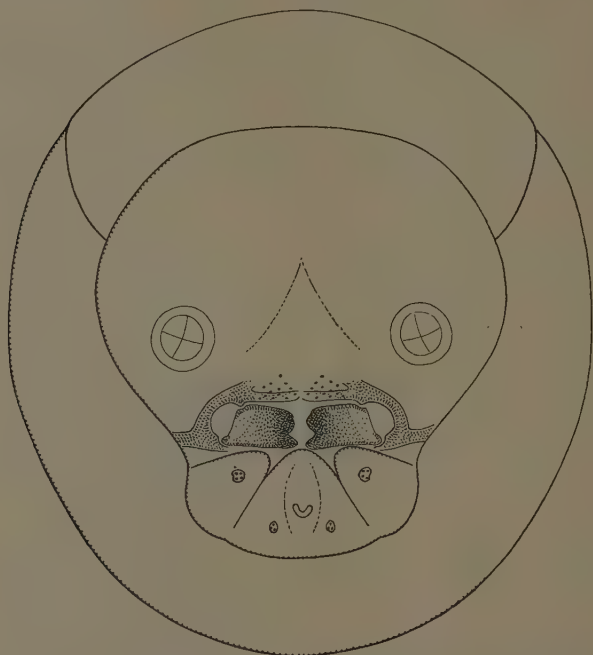


Fig. 4.—*Evania appendigaster*: Head (and first body segment) of mature larva to show arrangement of mouth parts, cephalic skeleton, etc. ($\times 38$).

Larvae. Solitary Hymenopterous larvae in oöthecae of cockroaches (*Periplaneta americana*, *P. australasiac* (F.), *Blatta orientalis* L., etc.). Mandibles either triangular and multi-toothed, as in the first instar, or gauntlet-shaped and tridentate as in the second and third instars, or massive sub-triangular and bidentate with one narrower curved terminal tooth and one larger blunter denticle, as in the fourth and fifth instars.

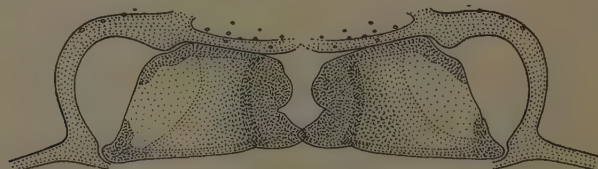


Fig. 5.—*Evania appendigaster*: Cephalic skeleton of mature larva ($\times 98$).

Adults. Hymenopterous parasites *ex* above hosts with the abdomen laterally compressed and attached high up on the propodeum (fig. 6). Forewings (fig. 7, a) with a distinct costal cell and hind wings (fig. 7, b) with a pronounced anal lobe.

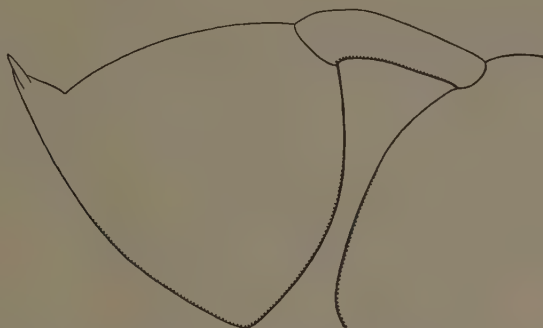


Fig. 6.—*Evania appendigaster*: Abdomen of female, lateral view, to show attachment high up on propodeum ($\times 17$).

Evaniids and the Biological Control of Cockroaches.

It was pointed out in the first paper of this series that the biological method of control might prove to be a useful way of dealing with the cockroach problem, either by itself alone, or in combination with other measures. Insecticides like DDT and γ BHC, etc., are of course valuable aids to control, but the inaccessibility of cockroaches is a difficulty which cannot be overcome by chemical methods however well they may be applied. Since the parasites and predators

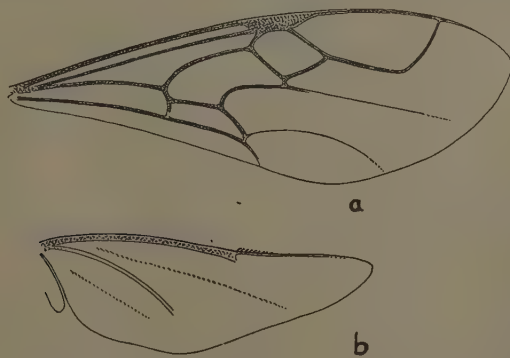


Fig. 7.—*Evania appendigaster*, wings: a, forewing, to show the costal cell; b, hind wing with distinct anal lobe ($\times 12$).

are not hampered by problems of approach, it would appear from the present investigation that they can and do exert a considerable effect on the cockroach population especially in areas where they are well-established. The first of these parasites to be investigated by the present author was the Eulophid, *T. hagenowii*. This species, with a parasitism rate in cockroach oöthecae of from 15–57 per cent., undoubtedly exercises a most beneficial effect in reducing the numbers of its host, mainly *P. americana* (also *P. australasiae*, *P. fuliginosa* (Serv.), *B.*

orientalis, *Eurycotis floridana* (Wlk.), *Parcoblatta* sp., and *Neostylopyga rhombifolia* (Stoll)). When it is also borne in mind that the rate of multiplication of this parasite, with up to six generations a year in some areas, is many times that of its host (less than one generation a year) it will be apparent that the reduction in the numbers of the cockroach population effected by this one parasite alone must be very considerable indeed. The part played by *Evania appendigaster* in the biological control of cockroaches is also an important one. Its parasitism rate in the Middle East area, amongst oöthecae of *Periplaneta americana*, ranges from 20-29 per cent., a somewhat lower rate than that of the Eulophid, but still quite a high degree of parasitism. This species also, with several generations a year, breeds more rapidly than its host, thus increasing its effectiveness as a controlling agent very considerably. It is also more efficient than *Tetrastichus* in the sense that one egg laid in an oötheca will normally produce a larva which by itself will completely destroy the full complement of eggs in the egg-capsule of its host. To achieve the same effect, *T. hagenowii* must lay 30-40 eggs (on the average, *hagenowii* females produce about 100 offspring and attack 2-5 oöthecae—Roth & Willis, 1954). There are, however, certain other factors to be taken into consideration in assessing the relative merits of the two parasites. These are the somewhat slower development of *E. appendigaster* as compared with *T. hagenowii*, the greater hardiness of the latter, and the fact that neither parasite is entirely specific in its choice of hosts. As far as hardiness is concerned this is a point which may not be relevant in the field, but it was noticed in laboratory experiments that larvae of *T. hagenowii* were seldom, if ever, affected by the opening up of the host oötheca for inspection, whereas larvae of *E. appendigaster* so treated reacted very unfavourably, and many of them died. Dryness is another factor to be taken into consideration, since quite a number of both parasites and hosts failed to emerge because of apparently over-dry conditions. However, all these factors being duly considered, the fact remains that *E. appendigaster*, in the areas where it is well-established, is a valuable parasite from the biological control point of view. Between them, the two species, *T. hagenowii* and *E. appendigaster*, appear to be capable of destroying more than 50 per cent. of the cockroach population. Whether they actually do so in any one area is not quite certain, because their distribution, although theoretically widespread, does not always coincide in actual practice. For example, both parasites have been recorded from the West Indies, yet no larvae or adults of *E. appendigaster* were found in the Trinidad material. In that island, *T. hagenowii* appears to be the dominant parasite. The reverse holds true for Saudi Arabia, where the majority of the collections from Jedda were parasitised only by *E. appendigaster*. This is a matter, however, which could be rectified by the introduction of both parasites in reasonable numbers into any area where biological control measures might be envisaged. Although the biological control of cockroaches has not hitherto been attempted to any great extent, the experience of Zimmerman (1948), who tells us that the accidental introduction of the egg-parasite, *Comperia merceti* (Comp.), with its subsequent distribution to various parts of Honolulu, successfully controlled the cockroach, *Supella supellectilium* (Serv.), is a heartening pointer to what may conceivably happen with *T. hagenowii* and *E. appendigaster*. There are, of course, other parasites, including further species of *Evania*, the unusual Coleopteron *Rhipidius pectinicornis* Thnbg. and the Ampulicid predators which attack adult cockroaches, still to be investigated. If suitable material can be obtained it is hoped that further work will be carried out on these very interesting species in due course.

Host Records and Distribution of Evaniids.

(in the restricted sense of Townes (1949) and Casskey (1951))

An up-to-date and fully documented list of all the Evaniids so far recorded from cockroaches is given below. ~~They~~ ^L are mainly tropical in distribution but at the members of this family

least eleven species occur in the Nearctic Region and two have been taken in Britain. All of them are parasites of the egg-capsules of cockroaches.

believed to

***Evania appendigaster* (L.) (syn. *E. laevigata* Ol.).**

Recorded from the following hosts and countries:—*Periplaneta* sp., Fiji (Lever, 1946); *P. americana*, Jamaica (Gowdey, 1925), Palestine (Klein, 1933), Hawaii (Swezey, 1929); *P. australasiae*, Hawaii (Swezey, 1929); *B. orientalis*, Hungary (Kadocsa, 1921), Egypt (Alfieri, 1914); *Cutilia soror* (Brunner), Hawaii (Swezey, 1929); *N. rhombifolia*, Hawaii (Swezey, 1929). As regards U.S.A., Townes (1949) states that this parasite is common in Arizona and in the cities of the Gulf and Atlantic States as far north as New York.

***Evania dimidiata* Spin. (syn. *E. abyssinica* Westw.).**

Recorded from a Blattid, probably *B. orientalis*, in Egypt (Alfieri, 1914).

***Evania impressa* Schlett.**

Recorded from *Periplaneta* sp. in Fiji (Lever, 1946).

***Evania sericea* Cam.**

Recorded from *P. americana*, *P. australasiae*, *C. soror* and *N. rhombifolia* in the Hawaiian Islands (Swezey, 1929); *Periplaneta* sp., Fiji (Lever, 1946).

***Evania subspinoso* Kieff.**

Recorded from *Periplaneta* sp. in Fiji (Lever, 1946).

***Prosevania punctata* (Brullé) (syn. *Evania punctata*).**

Recorded from *B. orientalis*, Spain (Cros, 1942); *Blattella germanica* (L.), Turkey (Fahringer, 1922); *P. americana*, Palestine (Klein, 1933). As regards U.S.A., Townes (1949) states that this species is a native of the Mediterranean region but is now naturalised in the cities of the eastern United States.

***Zeuxevania splendida* (Costa).**

Recorded from *Loboptera decipiens* (Germ.) in France (Genieys, 1924).

***Hyptia reticulata* (Say).**

Recorded from *Parcoblatta pensylvanica* (Deg.) in U.S.A. (Rau, 1940).

***Hyptia thoracica* (Blanch.).**

Recorded from *P. pensylvanica* in U.S.A. (Townes, 1949).

***Brachygaster minutus* (Ol.).**

Recorded from *Blatta orientalis*, *Blattella germanica* and *Ectobius lapponicus* (L.) in Europe, particularly Hungary (Kadocsa, 1921, and Nat. Hist. Mus. records for *Ectobius* in England).

Summary.

A detailed account of the biology and development of an important parasite of cockroaches, the Evaniid, *Evania appendigaster* (L.), is given. Adult and immature stages of the parasite were obtained and studied from collections of

oöthecae of *Periplaneta americana* (L.) made in Saudi Arabia, some 25-29 per cent. of which were parasitised. There are at least three or four generations a year, a rate of multiplication which gives the parasite a very decided advantage over its much slower-breeding host (*P. americana* takes at least a year or more to pass through one generation).

The Evaniids are a neglected group of parasitic Hymenoptera and so far very little has been published about them.

Adult parasites emerged from the material collected in Saudi Arabia in or about February, May and October. Oviposition in this species is carried out in a rather peculiar manner. The female lies on her side and, with legs braced against the oötheca, penetrates the tough integument of the egg-capsule after about half-an-hour's hard labour. Only one egg (hymenopteriform type) is laid in an oötheca, and the larva which develops from it is solitary in habit, and completely devours all the eggs in the egg-capsule of its host. The number of larval instars was determined by an examination of the cast skins in the residual material of parasitised oöthecae, a task rendered easier by the very distinctive mandibles of the various stages. Altogether there are five separate larval instars, the first easily identified by the serried arrangement of small denticles on the mandibles. The mandibles of the next two stages are tridentate and shaped like a gauntlet glove, while those of the last two instars are sub-triangular and bidentate. The mature larva is described in detail, and recognition characters for eggs, larval instars and adults are provided.

An appraisalment of the value of the two important parasites, *Tetrastichus hagenowii* (Ratz.) and *E. appendigaster*, in the biological control of cockroaches is made. It is pointed out that *T. hagenowii* in certain areas destroys from 15-57 per cent. of the eggs of its host and *E. appendigaster* 25-29 per cent. Both the former, with up to six generations a year, and the latter, with three or more, multiply much more rapidly than their host. Between them the two parasites appear to be capable of destroying up to 50 per cent. of the cockroach population.

The host records and general distribution of five species of *Evania*, one each of the related genera *Prosevania*, *Zeuxevania* and *Brachygaster*, and two of *Hyptia*, all of which parasitise the oöthecae of cockroaches, are fully documented. *E. appendigaster* itself has been recorded from the main species of cockroach, *P. americana*, *P. australasiae* (F.), *Blatta orientalis* L., *Cutilia soror* (Brunner) and *Neostylopyga rhombifolia* (Stoll), and its distribution ranges from Europe (Hungary) and the Middle East (Palestine) to the Pacific (Hawaii and Fiji), America (Gulf and Atlantic States), and the West Indies (Jamaica).

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A NEW METHOD OF EXTRACTING ARTHROPODS AND
MOLLUSCS FROM GRASSLAND AND HERBAGE
WITH A SUCTION APPARATUS.

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(PLATE VI.)

E.M.N.

The sampling of arthropods from low-growing plants, grassland, crops or similar habitats has usually been done by sweeping, or by taking away whole plants or their parts, from which the insects are subsequently removed; or by removing the upper layer of the soil with the plants growing on it.

All these methods may, at times, be appropriate; but there is a need for a simple, more universal and accurate method which gives consistent, known and high extraction rates for a wide variety of arthropods living in herbage. This applies particularly to the fauna of rough, matted grassland with its associated weeds and soil surface. For these, sweeping may be useless and liable to large personal errors, while the removal of parts of the habitat may be too laborious, or undesirable.

The portable apparatus described below has been developed for extracting efficiently the free-living arthropods from small areas of such a habitat without the need to remove plants; the method may also be suitable for other habitats such as the surfaces of tree trunks, leaves of trees or shrubs, or for litter.

Preliminary tests with several groups of arthropods in grassland have shown the worth of the method. For particular species or in other habitats, restandardisation and, perhaps, modifications to the apparatus would be necessary.

The Apparatus.

The suction unit is a Wolf Portable Electric Blower (Type NWBE) running on 220-250 volts A.C.; motors for other voltages, both A.C. and D.C., are also obtainable. The apparatus (Pl. VI, fig. 1, text-fig. 1) delivers approximately 60 cu. ft. of air/min., and the air velocity at the nozzle that was used (diam. 1.2 in.) is about 70 m.p.h.

A wooden annulus is fitted round the intake of the blower and a metal cylinder (10 in. long, 5.5 in. internal diameter) is fastened to this. A lid of paxolin with a central aperture is held on to the top of this cylinder by two spring clips. A short length of brass pipe projects from the centre of the lid and the rubber bayonet-type connector on the end of the flexible hose (supplied with the Wolf blower) fits on to it. The long rubber detachable nozzle supplied with the blower was cut down to a length of 7 in.; the internal diameter of the distal end is then just over 1 in.

A 7-in.-long collecting bag of nylon (mesh 18 per 5 mm.) sewn on to a flat metal ring, rests on the rim of the metal cylinder. The paxolin lid, with a rubber washer on the inside, is clipped over this and makes an airtight seal (fig. 1).

The apparatus weighs 15½ lb. and is easily carried and handled.

Operation and Standardisation.

Sampling routine.

A small area of rough grassland was delimited by a metal cylinder (12 in. high, 12 in. diameter) whose lower, sharpened edge was forced into the ground (Pl. VI,

fig. 2). With the blower in operation, the nozzle of the apparatus was drawn over the upper parts of the vegetation within this cylinder, flowering heads and other upright parts actually being drawn repeatedly in and out of the nozzle. The nozzle was then pushed down among the bases of the grass and twice worked

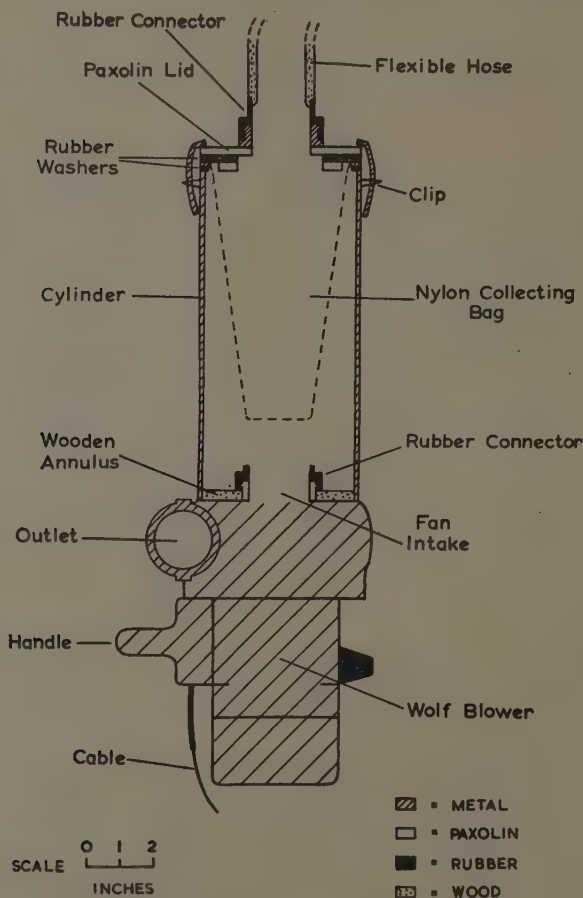


Fig. 1.—Semi-sectional diagram showing construction of the apparatus.

to and fro systematically over the whole area within the cylinder. This took from two to three minutes. The apparatus was then switched off, a cover placed over the cylinder in the grass and the collecting bag, containing a mass of grass blades, dead leaves, detritus and small animals, was emptied into a bottle. After an interval of about two minutes the process was repeated; this usually took slightly less time than the first, owing to the previous disturbance, and fewer animals were extracted than in the first operation. These two extractions made the sample and were put together in a bottle and taken back to the laboratory where the

animals were at once sorted by hand from the débris, every effort being made to ensure complete clearance. Avoidance of delay in sorting minimises errors due to possible fresh emergences or deaths and desiccation. For relatively large sluggish insects, or where only one fairly obvious group of animals was being collected (as with Heteroptera), it was sometimes practical and convenient to sort on a white sheet in the field immediately after taking the sample; each complete sample then took about 15 to 20 minutes.

The Heteropterous fauna of the flowering heads of grasses was sampled by drawing the grass heads in and out of the nozzle of the apparatus and flicking the flowers with the fingers as they came out. Using this method, two operators, one actually sampling and the other emptying the collecting bag and recording, can sample a grass head in less than a minute. For more active animals, such as Diptera, various modifications would be necessary. For Auchenorrhyncha or flies such as *Meromyza*, a muslin sleeve, fitted to the top of the metal cylinder that delimits the sampling area, would prevent their escape, and sampling could then be done through this sleeve. With even more active insects, such as *Calliphora*, it would probably be best to sample at night, or at least to place out muslin-covered cylinders during the night when the flies are resting on the grass. Estimates of the total population of such animals should be made during their inactive period (in this case, at night), otherwise a large proportion of the population will be in the air.

Hard-bodied animals such as Coleoptera and Diplopoda appear to be undamaged when collected in the apparatus. Softer insects are sometimes killed; this is not commonly a disadvantage and the material was always found to be in a condition permitting identification. If, however, undamaged and live animals are required, the diameter of the flexible hose and the collecting cylinder with its nylon bag could be increased, thus reducing the air speed through the apparatus.

The apparatus was frequently used on well over a quarter of a mile of cables; there is little doubt that this distance could be greatly extended with suitable light (e.g., P.V.C. sheathed) cable.

Routine in assessing efficiency of the method.

To assess the efficiency of the method in each particular type of habitat it was necessary to find the number of animals remaining within the habitat inside the metal cylinder after sampling.

In carrying out such a test, the first and second parts of a sample taken as described above were placed separately in two jars and the animals in each part sorted and counted separately. The two sets of data were subsequently pooled.

All the grass and débris above the soil and a small part of the surface soil and superficial grass roots within the area delimited by the cylinder were removed by hand or with a small trowel. This material was put in a jar and at once sorted in the laboratory to remove all the arthropods and molluscs that remained after suction.

Results.

The typical habitat sampled was about three-quarters grass (mostly *Dactylis*, *Holcus* and *Poa trivialis*), with occasional weeds (e.g., *Plantago*, *Urtica*, *Cirsium*) making up the remaining quarter. The coverage of the soil was very high, scarcely any bare ground being visible. A matted cover of leaves and stems at the base of the grasses varied up to 2 in. in thickness.

Thirty samples were taken between July 1954 and July 1955. In one there was an extremely high proportion of ants, and these were omitted from the total. The mean unweighted percentage extraction was then obtained: this together with other parameters is shown in Table I.

TABLE I.
Extraction rates and relevant data for the major groups taken from rough grassland.

Group	Mean % extraction	S.D.	No. extracted	No. of batches of 5 samples	Coefficient of variation
Collembola - Arthropleona	99.3	1.22	1010	6	1.23
" - Symphyleona	100.0	0	88	6	0
Thysanoptera	97.5	3.11	137	3	3.19
Hemiptera - Auchenorrhyncha	98.1	2.32	216	6	2.37
" - Aphidoidea	99.8	0.41	276	6	0.41
" - Heteroptera	98.9	2.72	61	6	2.75
Coleoptera - Staphylinidae	92.2	12.95	119	6	14.04
" - Pulidae	97.4	3.70	215	6	3.80
Other Coleoptera	92.4	10.50	100	6	11.46
Coleoptera - larvae	70.3	27.58	49	6	39.24
Hymenoptera - Parasitica	97.0	7.42	72	6	7.65
Diptera - adults	99.2	2.04	115	6	2.06
" - larvae & pupae	75.5	12.88	364	6	17.06
Isopoda	98.2	3.12	93	6	3.18
Acarina	90.2	13.63	1262	6	15.11
Araneida	97.4	2.13	160	6	2.18
Diplopoda	84.1	14.57	34	6	17.32
Chilopoda	66.7	42.17	41	6	63.25
Mollusca	94.7	6.22	189	6	6.57

The data were divided into the major groups of animals and every five consecutive samples were pooled.

There is a consistent and very high extraction rate of between 90 and 100 per cent. in 15 of the 19 groups. But millipedes, centipedes, Dipterous larvae and pupae and beetle larvae showed a lower extraction rate and the method is not entirely suitable for them.

Some groups of which very small samples were taken also showed a lower extraction rate and are not included in the Table, for example, Carabidae, 87.5 per cent., 7 out of 8 extracted; Lepidopterous larvae, 71.4 per cent., 10 out of 14 extracted.

There was no significant difference between the results from three pairs of operators; but the greatest source of error is obviously in sorting rather than in the use of the apparatus. Some groups, *e.g.*, mites, thrips or Collembola, might show a considerable variability due to this source of error, for they can easily be

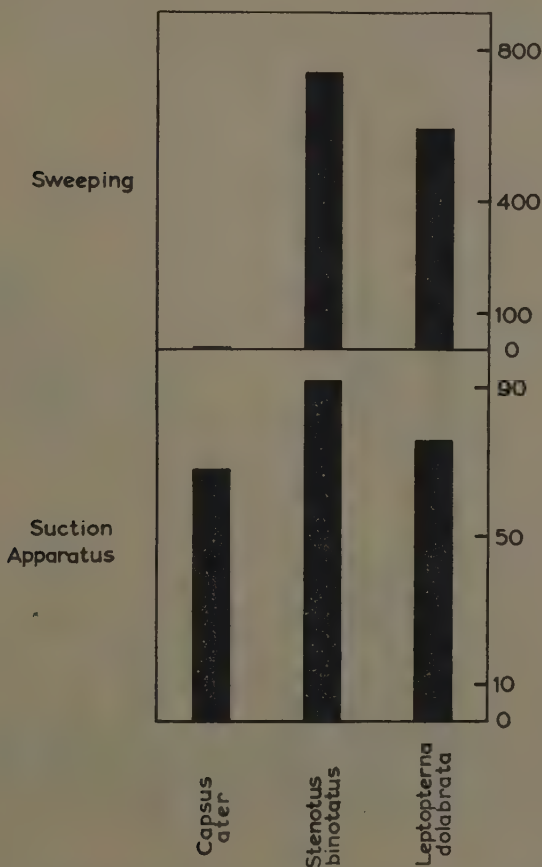


Fig. 2.—The total numbers of nymphs of three species of MIRIDAE collected in the same area by the suction apparatus and by sweeping.

missed. This, however, is likely to occur with almost any method, and we think that very few animals indeed were missed in our sorting.

Another source of error, particularly when sampling damp habitats, is the possibility of small insects sticking on to the inside of the flexible hose, though there was no indication of serious sticking in our samples, which were taken in reasonably dry weather. The method, in common with most others, is unsuited for use when habitats are very wet.

This method of sampling by suction is obviously applicable only to insects living on the surface or in the more accessible crevices of plants and soil. Then, as shown in Table I, an extremely high extraction rate is obtained. Indeed, surface dwellers could be defined conveniently as those inhabiting that part of the soil removed by a standard suction apparatus.

The suitability of the method for Aphids depends on the species concerned. Thus it is extremely suitable for those Aphids which, like *Megoura viciae* Buckt., fall off the food-plant on the slightest touch and have, in the past, been difficult to sample quantitatively. Indeed, most Aphids in Table I were *M. viciae* from vetch. But for species which are attached firmly to stems by their stylets, e.g., *Aphis fabae* Scop. on beans and *Brachycaudus cardui* (L.) on thistles, the method is quite unsatisfactory.

In general, the suction method has four distinct advantages over sweeping, namely:—

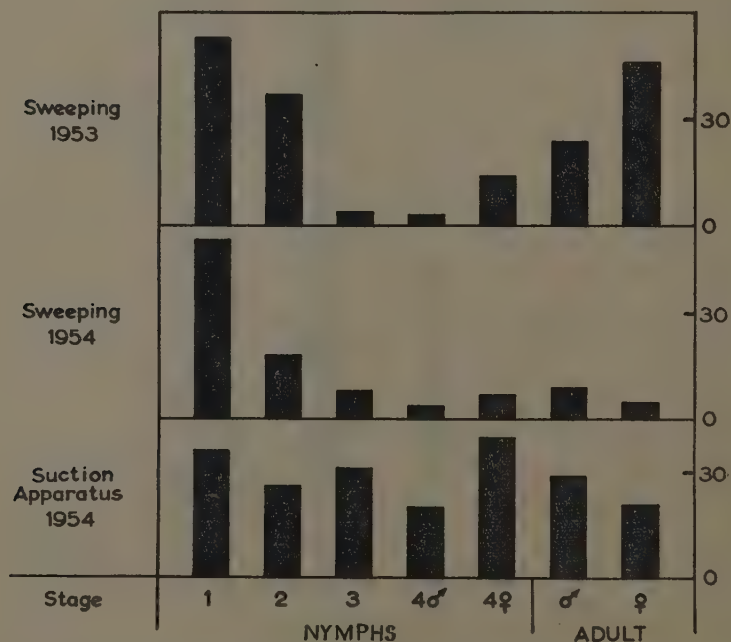


Fig. 3.—The total numbers of each stage of *Nabis limbatus* collected by the suction apparatus and by sweeping.

1. It gives an almost complete extraction of the population of certain groups from a known area.
2. It is less liable to personal error.
3. It collects from otherwise inaccessible places, *e.g.*, between the bases of grass stems.
4. It permits the selection of places within the habitat, *e.g.*, grass heads may be sampled separately from the bases of the same plants.

It is interesting to compare the captures of the nymphs of three species of MIRIDAE (Heteroptera) as collected by sweeping and suction from the same patch of grassland over the same period (Southwood, 1955). From suction samples, nymphs of *Capsus ater* (L.) (whose earliest instars were previously unknown) and nymphs of *Leptopterna dolabrata* (L.) and *Stenotus binotatus* (F.) were almost equally abundant (fig. 2). However, in samples taken by sweeping, *S. binotatus* and *L. dolabrata* were extremely abundant, but only two nymphs of *C. ater* (and these in the last instar) were taken.

When the flowering heads of two grasses, *Dactylis glomerata* and *Holcus lanatus*, were sampled separately with the suction apparatus, only *L. dolabrata* and *S. binotatus* were taken. When the matted bases of the grasses were similarly sampled, *C. ater* was found. Sweeping fails completely to give evidence of this stratification.

Similarly, many first- and second-instar nymphs of *Nabis limbatus* Dahlb. (Heteroptera, NABIDAE) were taken by sweeping a fixed area each week throughout the summer, but those in the third and fourth (last) instars were taken only in very small numbers (fig. 3). Samples over the same area with the suction apparatus included relatively many more examples in the third and fourth instars. It may be supposed that nymphs in the earlier instars frequent the tops of the grasses and those in the later ones the bases. The length of the later instars and of the adult life is also longer than that of the earlier stages, which thus increases the numbers available for sampling over the season. This would account for the taking, in the true sample collected with the suction apparatus, of the larger numbers of fourth-instar nymphs and adults, as compared with the numbers in the earlier instars (fig. 3).

Summary.

A new method of extracting free-living arthropods and molluscs from rough, matted grassland has been developed which gives extraction rates that are usually above 95 per cent. Its advantages over other known methods, especially sweeping, are that it is less liable to personal error, collects from otherwise inaccessible places in the habitat, permits selection of places within the habitat (*e.g.*, tops and bases of grass) and does not depend on removal of parts of the habitat.

The apparatus consists of a light, portable but powerful electric suction pump of standard pattern, with a special compartment, containing a small collecting bag, fitted between the fan intake and the flexible hose that carries a nozzle 1 in. in diameter, which is inserted into the grass. It can be operated on several hundred yards of cable.

The sample obtained comprises a mass of plant matter and detritus from which the animals are easily sorted by hand in the laboratory.

The method is unsuitable for subterranean arthropods or for insects living inside or otherwise firmly attached to plants. But it should be possible to use it for a variety of habitats other than grassland.

Acknowledgements.

Our thanks are due to Mr. L. A. Speed, of the R. & D.E., Cardington, for help with the construction of the apparatus, and to Miss P. Boutwood, Mr. A. J. Cockbain, Mr. C. Carter and Mr. G. G. E. Scudder for assistance with collecting

and sorting. Mr. H. E. Goto, of the Imperial College of Science, kindly identified the Collembola.

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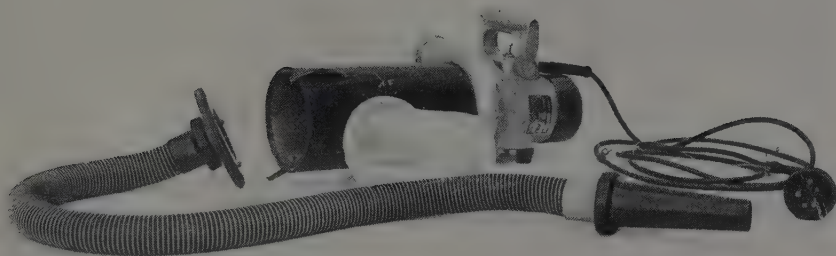
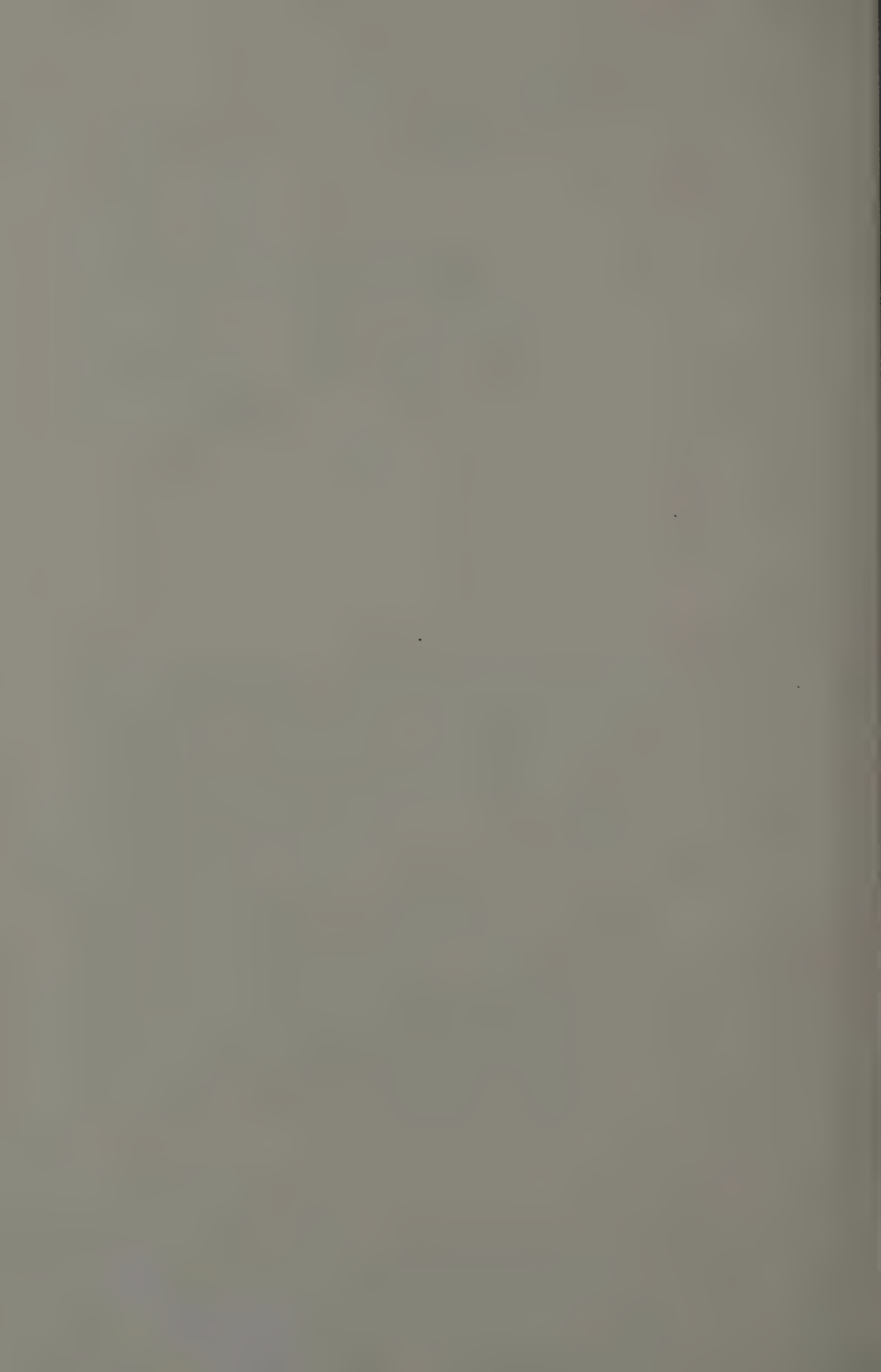


FIG. 1. The apparatus, with the suction hose and lid detached and the nylon collecting bag removed and displayed.



FIG. 2. The apparatus in use.



STUDIES OF BRITISH ANTHOMYIID FLIES.

VIII.—THE CARNATION FLY, *HYLEMYIA BRUNNESCENS* (ZETT.).

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The Carnation Fly, *Hylemyia brunnescens* (Zett.), occurs almost every year as a serious pest of various species of *Dianthus*. The larvae feed in blotch mines in the leaves and they also tunnel in the shoots and destroy the terminal buds. Bruneteau (1930) and Séguy (1932) recorded severe attacks in winter on carnations (*D. caryophyllus*) under glass, and carnations and pinks (*Dianthus* spp.) in borders are also frequently infested. Sweet william (*D. barbatus*) is regularly attacked and injury to plants grown for seed may be so severe that they are not worth growing to maturity. Bruneteau (1930) found the Carnation Fly on ragged robin (*Lychnis flos-cuculi*) and noted that it had been found by Hering on white campion (*Melandrium album*). Its occurrence on such common wild plants accounts for its frequency and its wide distribution. There has been no detailed study of the Carnation Fly in England, but Wilson (1950) stated that there were two generations a year in the open, with adults in spring and again in July, and that there were possibly three generations in glasshouses and frames.

In European literature, three species of Anthomyiid flies have been associated with injury to carnations, namely *H. brunnescens*, *H. cardui* (Mg.) and *H. fugax* (Mg.). It has been established (Miles, 1950) that *H. fugax* is a saprophytic species; its presence on attacked *Dianthus* spp. is, therefore, of no importance and may be ignored. The published accounts of the biologies of the other two species are incomplete.

Previous Accounts of Flies infesting *Dianthus* spp.

Bruneteau (1930) gave the first detailed account of the biology of *H. brunnescens*. He stated that the flies were active on warm days from spring until autumn, and that there were two ill-defined generations a year, with oviposition at the beginning and the end of summer. He further stated that he had seen the larvae in April, July and December and concluded that there were probably three generations a year under glass. He emphasised, however, that "les élevages sont très capricieux et je n'ai pu, malgré de grandes précautions, suivre la vie des mouches durant une année".

Bruneteau acknowledged that he had not studied the eggs sufficiently and did not describe them. He recorded that they were usually laid singly on the upper sides of the leaves, and observed that the larvae penetrated the epidermis and fed on the mesophyll. The mines were directed towards the bases of the leaves, and after feeding in the leaves the larvae entered the shoots. He gave a general description of the larvae but his illustrations did not show any specific characters. He stated that the winter was passed in the pupal stage, which lasted 20–48 days according to the temperature, and he gave the total length of life as two to two and a half months. He further stated that the flies began to emerge towards the end of March and lived from four to eight days.

It is evident that Bruneteau succeeded in rearing *H. brunnescens* for identification but his account of the biology has several omissions. He did not state the

times of the year when his observations were made and he did not show clearly whether he had observed the complete cycle from egg to adult.

Séguy (1932) described in detail the biologies of *H. brunnescens* and *H. cardui* on carnations. The account of *H. brunnescens* appears to be a transcription of that of Bruneteau and no new details are given, but the account of *H. cardui* is based on his own observations and it is discussed here because the biologies of the two species have so many common features.

Séguy stated that the adults of *H. cardui* were found from May to September on the herbage in cool, sunny situations at the edges of marshy places and in clearings in woods. He obtained eggs by dissecting a gravid female and found that they were covered by "une réticulation hexagonale peu marquée." He stated that the eggs were laid singly in the axils of the leaves, but he did not state that he had verified that the eggs on *Dianthus* spp. were similar in appearance to those he obtained by dissection. He described the larvae as feeding first on the parenchyma of the shoots and later, towards the end of the second larval stadium, migrating to the leaves where they fed on the mesophyll. He regarded this sequence of feeding sites and, more especially, the habit of tunnelling from the base of the leaf towards the tip, as characteristic of *H. cardui* and a reliable means of distinguishing it from *H. brunnescens*, whose larvae tunnelled in the opposite direction, that is, towards the leaf base. He illustrated larval characters of *H. cardui*.

Séguy's account of *H. cardui* has several notable omissions. Of special significance is the statement that "je n'ai jamais obtenu la mouche des larves âgées ou même de pupes", which indicates that he did not rear to the imaginal stage the larvae he described and did not, therefore, establish their identity. Further, the male genitalia that he illustrated were taken from dead pupae in the shoots of carnations "de provenance différente" and were not necessarily related to the females from which he obtained eggs or to the larvae he described. Since there was no continuous biological relationship uniting the sequence of stages that Séguy associated with *H. cardui* and since he did not succeed in rearing adults for identification, it is justifiable to conclude that he did not establish the life-cycle and habits of the species or its association with cultivated *Dianthus*. He also neglected to state the times of the year when he found the various stages on attacked carnations.

Balachowsky & Mesnil (1936) gave a short account of *H. brunnescens* which appears to combine some details from Bruneteau's account of that species with others from Séguy's account of *H. cardui*. They added some new observations, namely the size of the eggs and the univoltine character of the species, but they gave no information about the time of the year when eggs and larvae were to be found or when damage to plants occurred.

Life-history of *H. brunnescens* with Descriptions of Immature and Adult Stages.

The survey of the literature showed that the identity and life-cycle of the Carnation Fly had not previously been fully established nor the immature stages adequately described. The occurrence of injury on pinks and sweet william at Wye, Kent, afforded opportunities for observations that have been continued for several seasons, and material from other localities has permitted supplementary observations to be made. Only one Anthomyiid species has occurred on plants under observation and it has been identified as *H. brunnescens*.* Since this species has long been regarded in England as the Carnation Fly and since Séguy did not establish the existence of *H. cardui* on *Dianthus* spp., the use of *H. brunnescens* as the scientific name of the Carnation Fly has been maintained throughout this paper.

* Confirmed by Dr. F. I. van Emden, Commonwealth Institute of Entomology.

Eggs.

Eggs of *H. brunnescens* (fig. 1) are white and elongate oval, with one side convex and the opposite side rather flattened. The reticulation of the chorion is dominated by strong longitudinal ridges, somewhat irregular and broken like those of the eggs of Cabbage Root Fly (*Erioischia brassicae* (Beh.)) but thicker and less numerous. The eggs are truncate anteriorly and rounded posteriorly. Anteriorly



Fig. 1.—Eggs of *H. brunnescens* ($\times 26$).

there is a thick rim in which two strong ribs arise and extend along the flattened side for about a quarter of its length. At eclosion the larva breaks the chorion between the two short ribs and often leaves the smooth white vitelline membrane protruding through the hole. The average length of 34 eggs was 1.1 mm.

The eggs described above differ from those "d'un blanc éclatant, finement réticulés, et longs de 0.8 à 1 mm." that Balachowsky & Mesnil (1936) ascribed to *H. brunnescens*. There is, however, no doubt that the eggs found at Wye were those of *H. brunnescens*, because the cycle of development from egg to adult was completed under observation. It is a matter of some interest that the eggs of the Carnation Fly are similar to those of the Spinach Stem Fly, *H. echinata* (Séguy), but are distinctly larger (Miles, 1953).

Egg sites.

The eggs are laid singly on the upper surface of the leaves, as Bruneteau (1930) observed, but they are also found just as frequently in the axils of the leaves, the site described by Séguy (1932) for eggs of *H. cardui*. They are also occasionally deposited on the under surface of the leaves and on the stems between the nodes. They do not adhere to the plants and consequently they are easily dislodged by wind and rain. This probably has little effect on the survival of the larvae, because eggs on the leaves tend to be washed or shaken into the leaf axils.

Time of oviposition and duration of incubation period.

In 1954, eggs were first found in the open on 7th September and they were continuously present on plants under observation until 11th November. In 1955, eggs were first found in the open on 12th September. Flies reared in captivity in 1955 began ovipositing on 16th August and eggs were laid continuously until the last of the flies died on 26th September. Although plants under observation have been examined periodically throughout the year, eggs of *H. brunnescens* have been found only in autumn. That this is the usual and only time for oviposition has been confirmed by the behaviour of flies reared and maintained in captivity, for eggs have been found in the breeding cages only in late August and September.

The duration of the incubation period has not been accurately measured because the eggs laid by bred flies failed to hatch. Eggs of unknown age procured out-of-doors have hatched in from one to two weeks. This incubation period agrees with

the period of 12 days recorded by Bruneteau (1930) and with the period of eight or ten days observed by Séguy (1932) for eggs, stated by him to be those of *H. cardui*, from the leaf axils of carnations.

Larval feeding sites.

The larvae of *H. brunnescens* fed as leaf-miners and stem-borers in *Dianthus* spp. Their first feeding sites seemed to depend mainly on the positions of the eggs. Larvae from eggs laid on the leaves wandered at random over the leaf surface and finally penetrated the epidermis, possibly at the site of a small break in the tissue. Larvae from eggs in or near the leaf axils fed in the fleshy bases of the leaves or tunnelled into the stems and fed on the parenchyma. The difference in feeding habits that Séguy (1932) used to distinguish larvae of *H. brunnescens* from those of *H. cardui* proved invalid, because the larvae of *H. brunnescens* demonstrated both modes of feeding. Some larvae under observation fed entirely on leaf tissue, others fed exclusively within the stems or shoots, and some passed from leaves to shoots and *vice versa*.

The leaf-mines had a characteristic appearance that showed most clearly in the broad leaves of sweet william (*D. barbatus*). Larvae in all three stages devoured all layers of the mesophyll and the feeding tunnels were more or less clear and straight and usually followed the longitudinal axis. When a larva reached the leaf margin it usually turned and continued feeding in a parallel and contiguous channel. As the season advanced, the leaves became entirely covered with longitudinal feeding channels and practically all the mesophyll was devoured. The larvae left large quantities of dry, powdery, green frass in the mines, and the absence of excessive moisture and decomposition in the attacked leaves and shoots, which Séguy (1932) also observed, was probably an important factor contributing to the survival of the larvae that remained in the feeding sites throughout the winter. Leaf-mines in the narrow, grass-like leaves of carnation were similar to those in the leaves of sweet william, but were less conspicuous. Larvae that fed in the shoots killed the terminal buds and some lateral shoots, but the injury was not immediately visible because the low temperatures and high humidity of autumn and winter delayed the wilting and yellowing of the foliage.

Duration of larval life.

Larvae of *H. brunnescens* were found in the open at Wye from mid-September to mid-April, the earliest record being 11th September (1954) for first-instar larvae and the latest being 13th April (1953) for mature larvae in the shoots. Larvae taken in September and kept in an insectary became mature in November and burrowed into the sand provided for them. Larvae from eggs laid later in autumn developed more slowly, but the majority had finished feeding and left the plants by the beginning of January. Late larvae remained in the shoots and leaves throughout January and February. During the winter months, immature larvae fed intermittently. Feeding ceased altogether during frosty weather and continued when the temperature rose again to the level at which the larvae were active. Hering (1951) listed *H. brunnescens* among species having larvae that fed in winter when the temperature was only slightly above freezing point. Mature and immature larvae were unaffected by severe and prolonged cold weather. In January 1954 and again in January 1955, larvae taken after exposure to hard frost completed their development. All larvae under observation appeared to finish feeding by the end of February.

Bruneteau (1930) stated that *H. brunnescens* spent the winter in the pupal stage, but he did not record any dates of pupation. During two seasons at Wye, captive larvae were examined at weekly intervals to discover the length of larval

life and the times of pupation. In the winter of 1953-54, mortality among hibernating larvae was high in February and March and only about 5 per cent. (14 larvae) survived to pupate in early April. It appeared that my experience was similar to that of Séguy (1932) who found that "une larve extraite d'une feuille ou d'une galerie médullaire n'y reste pas si on l'y remet: elle s'enfonce dans la terre et meurt". In 1954-55, about 150 larvae were kept under observation and in that season the rate of mortality was low. None of the larvae had pupated before 20th March. There was then an unavoidable break in the weekly examinations and the larvae were buried out-of-doors to a depth of about 4 in. to provide them with conditions that were approximately normal. On 18th April, when they were next examined, about 80 per cent. had pupated. On 24th April, pupation was practically complete. A further examination on 11th May showed that no larvae remained.

These observations established that *H. brunnescens* hibernated as a larva and had a larval life of about six months. This appears to be a fixed biological characteristic, because larvae maintained at laboratory temperatures also failed to pupate on becoming fully fed. It was concluded, therefore, that the larvae of carnation flies found in glasshouses and frames in April by Bruneteau, Séguy and other workers belonged, like those found out-of-doors, to the overwintering generation.



Fig. 2.—Buccal armature ($\times 240$) of larva of *H. brunnescens*: a, first instar; b, second instar; c, third instar; v, ventral process.

Larval characters.

Larvae of *H. brunnescens* have a pair of bifid tubercles at the tip of the body and thus they resemble the larvae of the Cabbage Root Fly (*Erioischia brassicae*) and Wheat Bulb Fly (*Leptohylemyia coarctata* (Fall.)). When mature, they are about 9 mm. long, creamy white, stout and rather sluggish, with the body tapered towards the head and obliquely truncated behind. The head is small and retracted into the large prothorax. The mouth-hooks are stout and strong, with the surrounding integument lightly sclerotised. The antennae and maxillary palps are visible as small yellowish-brown spots immediately above the mouth-hooks on either side of a shallow median furrow. Each antenna consists of a basal ring and an almost spherical terminal segment. The maxillary palps are situated between the antennae and the mouth-hooks; each palp consists of sensory papillae surrounded by a sclerotised ring similar to the basal rings of the antennae.

The character of the buccal armature is shown in fig. 2. In the first instar, each mandible consists of one densely sclerotised mouth-hook with two large teeth anteriorly, a small tooth on the ventral surface and a tooth-like ventral process. In the second instar, the mandibles are stout, strong hooks, each with two subsidiary teeth on the ventral surface. In the third instar the mandibles are simple hooks, short, broad and heavily sclerotised. In each of the three larval instars, the buccal armature of *H. brunnescens* is similar to that of the corresponding instar of *H. echinata* (Miles, 1953).

The body segments are clearly defined and the integument is smooth and shining except where broken rows of rounded denticles form bands of varying width on the front edges and ventral surfaces of the segments. The anterior spiracles are small, inconspicuous and lightly sclerotised, and have 8-9 processes. The posterior spiracles are also small and lightly sclerotised. Each spiracular aperture has a well-defined peritreme, but the short pedicles that bear the spiracles are lightly sclerotised.

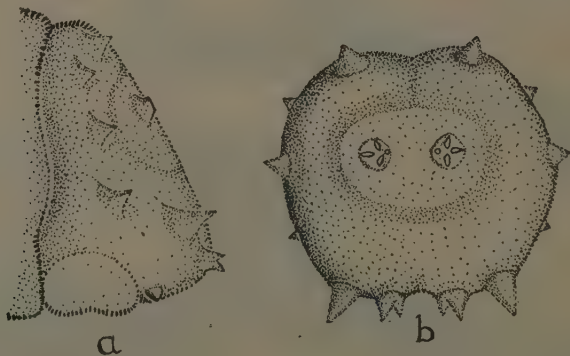


Fig. 3.—Eighth abdominal segment of larva of *H. brunnescens*:
a, lateral view; b, dorsal view.

The eighth abdominal segment is obliquely truncated dorsally and rounded ventrally, and the ninth segment, with the anus, is on the ventral surface. On the eighth segment, there are eight pairs of small, sub-conical tubercles, the arrangement of which is shown in figs. 3 and 4. The apical ridge bears a pair of large outer tubercles and an inner bifid pair formed by the fusion of each inner

apical tubercle with the adjoining ventro-apical tubercle. The supra-anal tubercles are more rounded than the rest.

The larval characters shown by Bruneteau (1930) are too general to be used for identification. Ségué (1932) gave some details of larvae (which he stated to be those of *H. cardui*) from carnations. He showed figures of the mouth-parts

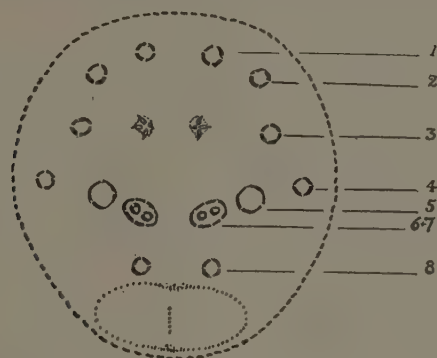


Fig. 4.—Diagram showing arrangement of tubercles on eighth abdominal segment: 1, dorso-central tubercles; 2, dorsal tubercles; 3, dorso-lateral tubercles; 4, lateral tubercles; 5, outer apical tubercles; 6 & 7, inner apical and ventro-apical tubercles; 8, supra-anal tubercles.

of the second and third larval instars, but they appear to be inaccurate, because differences in form between the mouth structures of succeeding instars result from the consolidation of component sclerites and not from the addition of new parts. The "dents locomotrices" and the anterior spiracles shown by Ségué are similar to those of *H. brunnescens*.

Pupation.

Pupation takes place within the larval integument, which hardens and darkens to form a brown puparium about 6 mm. long, barrel-shaped and showing some of the larval characters. Collinge (1912) recorded that the puparium was formed in the feeding site, but Bruneteau (1930) stated "je l'ai trouvée le plus souvent dans le sol, à une faible profondeur, au voisinage des racines". I have found at Wye that most of the larvae leave the host-plants for pupation in the soil, but small numbers, generally those developing late, remain within the plant tissue.

According to Bruneteau (1930), "la durée de la pupaison varie, selon la température, de vingt à quarante-huit jours". At Wye, in 1955, a total of 122 flies were reared in captivity. As already stated, pupation took place in late March and in April. Emergence began on 4th June and continued to 20th June. This indicated a pupal period of about 8 weeks and confirmed my more general observations of previous years. My observations thus support the longer period given by Bruneteau, but they also suggest that it is unlikely that *H. brunnescens* can complete its pupal development in as little as 20 days.

Duration of imaginal life.

In captivity, adults of *H. brunnescens* emerged from 4th–20th June 1955 with a peak period lasting from 6th–12th June. Observed dates of emergence of small numbers of flies in previous years were 30th May–4th June (1950), 2nd–9th June (1953), and 16th–26th May (1954). Bruneteau (1930) wrote of "la

sortie de terre commençant vers la fin mars", but gave no details of his observations. In the course of several years I have been unable to find any evidence of the occurrence of *H. brunnescens* in the imaginal stage before the middle of May.

Bruneteau also noted of the fly, "Sa vie est de quatre à huit jours, du moins au laboratoire". My experience did not confirm this. Some of the imagines that emerged in June 1955 remained alive until the last week of September, a period of nearly four months, and in previous years adults have survived for about three months.

The remarkable feature of *H. brunnescens* in captivity was the long pre-oviposition period. In 1955, no eggs were laid in the cages until the latter half of August, when the flies had already lived for 10-12 weeks. This could not be explained by reference to temperature and nutrition, since the temperature throughout the period was 70-80°F., and the flies fed freely on milk, sugar and water that had proved a satisfactory diet for several related species. The protracted pre-oviposition period seemed to be associated with a slow development to sexual maturity. Evidence for this was the healthy, vigorous condition of the flies after a prolonged period of captivity and the absence of any signs of senescence until the end of summer. Females showed no marked distension of the abdomen, a sign of maturing eggs, until about the beginning of August.

Supplementary field observations supported the view that *H. brunnescens* had a long imaginal life and only reached sexual maturity towards the end of the life span. Weekly examinations in 1954 and 1955 showed that eggs were absent during the summer from plants that became heavily infested in September and October in three consecutive years. In 1955, gravid females of *H. brunnescens* were captured on sweet william on 30th September. At this time a few eggs were present about the plants, but none had been observed during the summer months, although larvae from these plants had given rise to flies in the previous June, and, by October, signs of the presence of larvae were obvious. Séguy (1923) stated that adults of *H. brunnescens* were found from May to October. It now appears that this period represents the normal imaginal life of a single generation that emerges in May and June and that attains sexual maturity and produces eggs only in September and October.

Description of H. brunnescens.

The flies are 6-7 mm. long. Males have the head brownish and the thorax blackish-brown in front and yellowish-grey behind; the abdomen is strap-shaped with a narrow black median stripe and narrow black transverse bands on the front edges of the segments. Séguy (1923) and Karl (1928) state that the males are without long bristles on the ventral surface of the abdomen, but in fact bred specimens have long curved bristles ventro-laterally on abdominal segments 2-5. Females have the head yellowish-grey with a bright yellowish area above the lunule and two rows of post-orbital bristles more or less parallel with the upper margins of the eyes; the thorax and abdomen are yellowish-grey, the dark median stripe mentioned by Séguy and Karl being inconspicuous or absent in many specimens. In both sexes the antennae are black with three setae on the second segment and short pubescence on the arista; the proboscis, palpi and legs are also black. On the thorax, the acrosticals tend to be close together and short and fine, except for the pre-sutural pair, which are longer and stouter than the rest. The pre-alary seta is of moderate length but is shorter in males than in females. There are three sternopleural setae, the lower hind one being only slightly shorter than the upper one. The basal nervures of the wings, the squamae and the halteres are yellowish, and the costal spine is long. Males have a slight basal swelling on the second tarsal segment of the mesothoracic legs, which places them in the sub-genus *Delia* R.-D.; the chaetotaxy of the legs is not at present used in the identification of the species.

Annual Cycle of the Carnation Fly.

Observations on *H. brunnescens* indicate that there is only one generation a year in the open and under glass. The life-cycle has several features that appear to be unusual in phytophagous Anthomyiid flies, namely, that the adults live through the three hottest months of the year (June, July and August) before ovipositing, that the eggs are laid in autumn, that the larvae feed in winter and survive exposure to hard frost during the feeding period, and that hibernation takes place in the larval stage. These biological modifications fit the insects for life on the host-plants. The various species of *Dianthus*, *Lychnis* and *Melandrium* (Caryophyllaceae) make most of their vegetative growth in late summer and autumn, and they remain green and leafy throughout the winter. The larvae of *H. brunnescens* are relatively large and each larva devours about two square ins. of leaf tissue, and it is probable that it is only during the period of short days that there are sufficient leaves to sustain their attacks.

The retardation of sexual maturity is an appropriate biological device for transferring the larval feeding period to the winter months, when food is most abundant. In the case of *Leptohylemyia coarctata*, which also has winter-feeding larvae, the retardation of development occurs in the egg stage. This is appropriate for eggs laid in the soil. In *H. brunnescens* a different means is required, because the host-plants do not provide enough food for the larvae until late summer, and because the exposed position of the eggs would make a prolonged summer incubation period inappropriate. The univoltine character of the annual cycle might have been expected, for a species that is adapted physiologically to withstand the rigours of winter is not likely to be equally suited to exposure to summer weather. Hibernation in the larval stage might also have been expected in larvae that developed under conditions that induced slow metabolism.

Since the larvae of *H. brunnescens* occur only in autumn and winter, the records of attacks in summer require some explanation. Bruneteau (1930) stated "dans les serres . . . j'ai pu observer des larves en avril, en juillet et en décembre". Mr. F. H. Jacob, of the Ministry of Agriculture Plant Pathology Laboratory, Harpenden, has informed me of records of attacks by *H. brunnescens* in June (1945), July (1930) and August (1937) and Mr. V. W. Fowler, of the Royal Horticultural Society's Gardens, Wisley, has informed me of its occurrence in June (1945), July (1922 and 1942) and August (1944). My field observations suggest that these records may result from an error of identification and that injury to *Dianthus* spp. in summer arises from attacks by the Agromyzid fly, *Dizygomyza flavifrons* (Mg.),* which makes conspicuous silvery blisters on the leaves but does not attack the shoots. The mines made by *D. flavifrons* differ in position and appearance from those made by *H. brunnescens*. The former occur on both upper and lower surfaces of the leaves from May to November and the larvae feed only on the surface of the mesophyll, leaving it almost intact but dotted with minute fragments of black frass. Larvae of *H. brunnescens* devour the entire mesophyll and leave large quantities of dry, powdery, green frass between the upper and lower epidermis of the leaves.

This study of the Carnation Fly suggests that its occurrence as a pest may be forecast from an examination of the host-plants in the latter part of September. Since the oviposition period is limited to September and October, one or, at most, two sprayings with a suitable insecticide should give satisfactory control.

Summary.

The Carnation Fly, *Hylemyia brunnescens* (Zett.), which attacks cultivated species of *Dianthus*, has been studied in detail because the account given by Bruneteau (1930) was incomplete and that given by Séguéy (1932) of two flies

* Identified by Mr. H. Oldroyd, British Museum (Nat. Hist.).

(*H. brunnescens* and *H. cardui* (Mg.)) on carnations was also incomplete and the identities of the stages studied were not fully established. For the first time, the development of *H. brunnescens* from egg to adult has been completed under observation and bred adults have been maintained in captivity until eggs were laid. The eggs, larvae and adults are described.

Eggs were laid in autumn. Oviposition began in the field on 7th September 1954 and on 12th September 1955 and eggs were present until early November. The behaviour of flies in captivity confirmed that eggs were laid only in autumn.

The larvae mined in the leaves and shoots. Early larvae finished feeding in November and entered the soil. Late larvae remained on the plants and fed intermittently through January and February, whenever the temperature was suitable. Mature and immature larvae on the plants survived exposure to severe frosts. Hibernation took place in the larval stage in the soil or in the feeding sites. Pupation occurred from mid-March to mid-April and the pupal stage lasted about eight weeks.

The emergence period for 122 flies was 4th–20th June 1955 with a peak period from 6th–12th June; other observed emergence periods were 30th May–4th June (1950), 2nd–9th June (1953) and 16th–26th May (1954). Captive flies lived from two to four months. They were active during the summer but they did not reach sexual maturity and begin to lay eggs until late August. There is thus only one generation a year and the slow development to sexual maturity ensures that the larval period occurs in autumn when the foliage of the host-plants is freshest and most abundant.

Acknowledgements.

The writer thanks the Principal and Governors of Wye College for facilities for this study. She also gratefully acknowledges the interest and helpful criticism of Professor H. W. Miles. Thanks are also due to Mr. F. H. Jacob, Ministry of Agriculture Plant Pathology Laboratory, Harpenden, and to Mr. V. W. Fowler, The Royal Horticultural Society's Gardens, Wisley, for records of attacks by *H. brunnescens*.

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THE RESPONSE OF LARVAE OF THE LARGE WHITE BUTTERFLY (*PIERIS BRASSICAE* (L.)) TO DIETS OF MINERAL-DEFICIENT LEAVES.

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E.N.N

The direct approach to the study of the food requirements of insects is to rear them on artificial diets. This has, however, proved difficult in the case of those normally feeding on living plants, although larvae of the European Corn Borer (*Pyrausta nubilalis* (Hb.)) have been raised successfully in this way (Bottger, 1942; Beck & Lilly, 1949). Changes in the food value of leaves may, nevertheless, be readily induced under controlled conditions by growing plants in sand or water culture. Studies on insect growth and egg-laying rates on plant food raised in this way have been reported by Smith & Northcott (1951) (*Melanoplus mexicanus mexicanus* (Sauss.) on wheat), Dahms (1947) (*Blissus leucopterus* (Say) on sorghum); Wittwer & Haseman (1945) (*Heliothrips haemorrhoidalis* (Bch.) on New Zealand spinach); Haseman (1946) (greenhouse whitefly (*Trialeurodes vaporariorum* (Westw.)) on petunia and tomato; *Toxoptera* on wheat); Creighton (1938) (*Alabama* on cotton); Barker & Tauber (1951) (*Myzus persicae* (Sulz.) on *Tropaeolum*); Allen & Selman (1955) (*Phaedon cochleariae* (F.) on watercress). Similar work has also been carried out with a mite (*Tetranychus*) by Garman & Kennedy (1949) and by Rodriguez (1952). There are also numerous relevant field observations of uncertain value (reviewed by Allen, 1954).

Little is known concerning the response of leaf-eating caterpillars to the mineral nutrition of the food-plant, although Evans (1938) obtained indications that the carbohydrate and nitrogen content of the plant affected the rate of growth of *Pieris brassicae* (L.). He found that larvae fed on plants grown in the shade increased in weight more slowly and pupated later than those fed on plants grown in normal light. This slower rate of growth was probably related to the lower carbohydrate and nitrogen content of the plants grown in the shade.

In the present work it was found that larvae of *P. brassicae* were well suited to the study of the effect on insect development of mineral deficiencies in plants, since they are not limited to one species of food-plant, and are easily reared and handled.

Technique.

In the first experiment, larvae of *P. brassicae* were fed on leaves of cauliflower grown in sand culture at two levels of nitrogen; in the second experiment the diets consisted of leaves of turnip grown in sand culture with complete nutrients and with deficiency of nitrogen, phosphorus or potassium, respectively; in the remainder of the experiments leaves of watercress grown in complete nutrient or in iron-deficient water culture solutions were used. Apart from the differences between culture solutions (described below) the mineral-deficient and normal plants were grown under similar conditions and, in any one experiment, the different groups of plants were of similar ages. Before being supplied as food,

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the deficient plants were always showing characteristic symptoms. Fuller details of mineral-deficiency symptoms in watercress have been given by Allen (1954).

Culture solutions.

The composition of the "complete-nutrient" solutions (C.N.) were as follows:—

	Sand culture	Water culture
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	20.0 gm. per litre	0.50 gm. per litre
NaNO_3	14.4 " " "	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	5.0 " " "	0.09 " " "
KH_2PO_4	10.0 " " "	0.13 " " "
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.4 " " "	0.016 " " "

The solutions were made up in distilled water in all cases.

N, P and K deficiencies were induced by substitution of Cl for NO_3 , Cl for PO_4 and Na for K in the complete nutrient solution.

Plants used for studying the effects of N, P and K deficiencies were grown in sand culture. Fresh culture solutions were supplied weekly, tap water being given when necessary. Iron deficiency was induced by omission of ferric chloride from un aerated water cultures.

To reduce the variability of the material in any one experiment, larvae from one egg-batch were used whenever possible, since each batch is laid by one female only. The eggs used to produce larvae for most of these experiments were laid by adults reared in the laboratory using a technique similar to that of David & Gardiner (1952).

Each larva was caged in a glass tube (3 in. \times 1 in.) ventilated by means of a muslin-covered hole in the cork. Detached leaves were placed in the tube on a small pad of moist cotton-wool. Fresh leaves were supplied to the larvae *ad lib.*, being renewed daily except during the earlier part of experiment 1, when they were renewed on alternate days. As far as possible, leaves of similar physiological age were collected from the deficient and from the normal plants, care being taken to avoid senescent leaves. Larval weights were determined to the nearest milligramme and weighings were commenced as soon as the larvae were large enough to be handled without fear of injury.

Experimental conditions.

Temperature, humidity and light intensity were not controlled in the first two experiments, although in the second the larvae received light from 40-watt

TABLE I.

Experimental conditions.

Expt. no.	Temperature ($^{\circ}\text{C}$.)	Relative humidity (%)	Light intensity (foot-candles)	Day-length (hours)
1	Uncontrolled (summer)	Uncontrolled	Uncontrolled	Uncontrolled
2	Uncontrolled (autumn)	"	"	16
3 (a) & (b)	25	"	22	16
4	23	85-90	22	16
5	23	85-90	22	16

fluorescent tubes for 16 hours per day. In all later experiments, however, the tubes containing the larvae were housed in an incubator with controlled temperature, light intensity and day-length. Details are given in Table I.

Results.

The experiments may be divided into two groups according to whether they are concerned with the effects of deficiency of one of the three major plant-nutrient elements (N, P & K) or with deficiency of a trace element (Fe). The main effects have been found to be basically similar in both groups but an overall picture is gained most easily by first considering the experiments on the major elements before proceeding to the more detailed study of the effects of iron deficiency.

Statistical analyses were carried out on all numerical results.

Deficiencies of nitrogen, phosphorus and potassium.

Experiments 1 & 2.—Experimental details are given in Table II. Two experiments were performed, in each of which controls consisted of comparable groups of larvae fed on complete-nutrient leaves throughout.

All the diets composed of mineral-deficient leaves had a marked effect on the growth of the larvae, significantly reducing larval weights as compared with the control groups, and significantly lengthening the time taken to complete the larval period (see figs. 1 & 2).

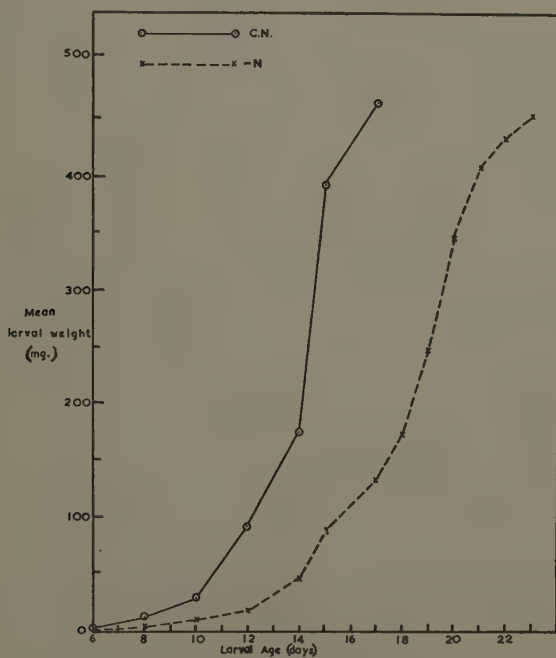


Fig. 1.—Growth curves for whole larval period on diets of complete-nutrient and nitrogen-deficient leaves, respectively. (Expt. 1.) In this and all other experiments, weighings were begun as soon as larvae were large enough to be handled without fear of injury.

The effect of the deficient diets on mortality rates was only noteworthy in the nitrogen-deficient group of experiment 2 (see Table III). In this case only one larva out of the total of 14 remained alive after 13 days on nitrogen-deficient diet as compared with the relatively low death rates in the other groups. The

TABLE II.

Data for experiments 1 & 2.

Expt. no.	Diet	Period on diet	Replication
1	-N	Hatching to pupation	x15
2	-N	" " "	x14
	-P	" " "	x14
	-K	" " "	x14

effect here was probably accentuated by low temperatures prevailing at the start of the experiment.

The relative growth rates of the different groups of larvae were also compared. The relative growth rate may be defined as the rate of increase in weight at any given time divided by the actual weight at that time. If the logarithms

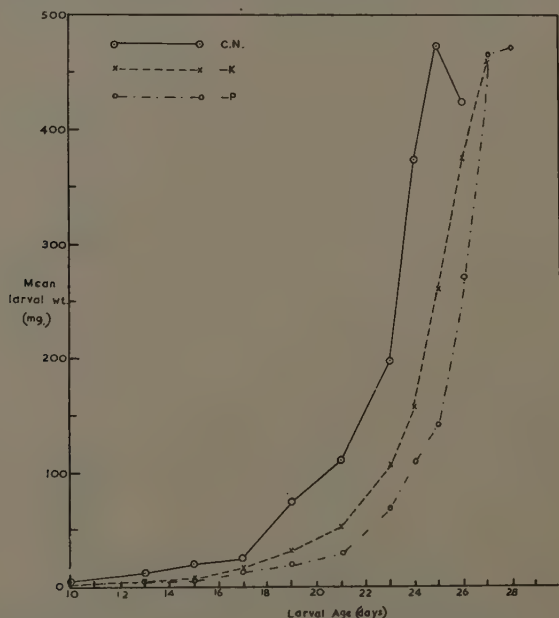


Fig. 2.—Growth curves for whole larval period on diets of complete-nutrient leaves and leaves deficient in potassium and phosphorus, respectively. (Expt. 2.)

of the mean weights are plotted against time they are found to lie approximately on a straight line. The relative growth rate as defined above is proportional to the slope of this line, which is represented by the regression coefficient " b ". Thus, the relative growth rates of different groups may be compared by statistically analysing the differences between the linear regression coefficients of \log_{10} weight on time.

TABLE III.
Larval mortality rates.

Diet	No. of larval deaths in expt. 1	No. of larval deaths in expt. 2
C.N.	0 out of 15	4 out of 14
-N	1 " " "	13 " " "
-P	—	5 " " "
-K	—	4 " " "

The regression lines for the control and nitrogen-deficient groups in experiment 1 are shown in fig. 3. The relative growth rates of the two groups differed significantly ($p < 0.001$).

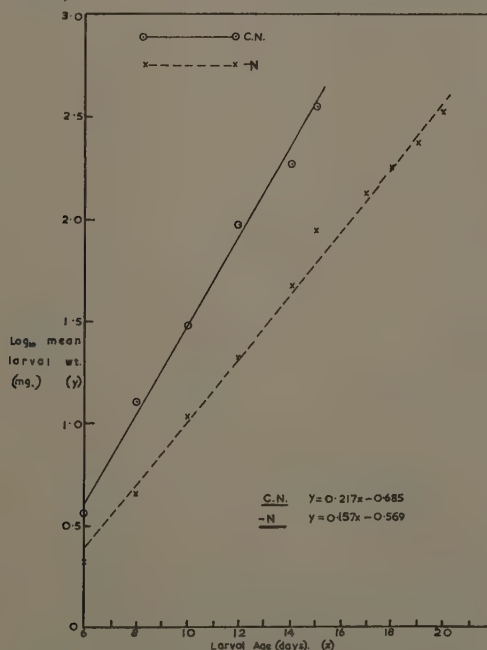


Fig. 3.—Comparison of relative growth rates of larvae reared on diets of complete-nutrient and nitrogen-deficient leaves, respectively. (Expt. 1.)

In experiment 2, all but one of the larvae in the nitrogen-deficient group died during the early part of the experiment. No significant difference was shown between the relative growth rates of the control (C.N.), phosphorus-deficient and potassium-deficient groups.

TABLE IV.

Data for experiments 3 & 4.

Expt. no.	Diet	Period on diet	Replication
3a	-Fe	Hatching to 9th day	x20
3b	-Fe	6th day to pupation	x10
4 group A	-Fe	Hatching to 13th day	x12
group B	-Fe	6th day to 13th day	x12
5	-Fe	Last 2 larval instars	x4

Iron deficiency.

Three experiments on the influence of larval diets of iron-deficient watercress leaves will be described. In these the adverse effects on larval development already recorded were again observed. In addition, the relationships between the age of the larvae fed on the leaves and the severity of the effects produced

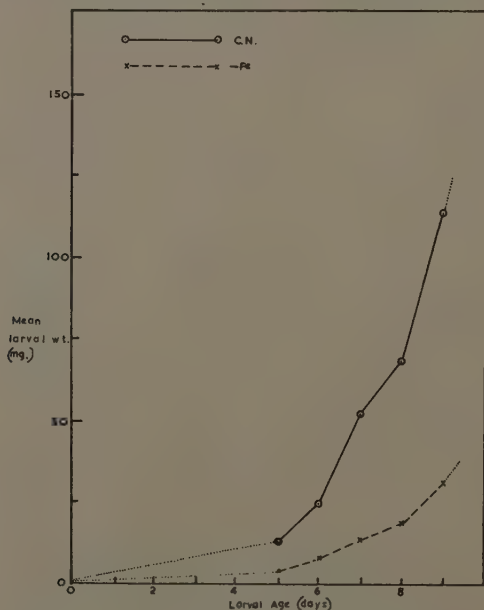


Fig. 4.—Growth curves from time of hatching to day 9 of larval life on diets of complete-nutrient and iron-deficient leaves, respectively. (Expt. 3a.) Weighings were begun as soon as larvae were large enough to be handled.

was investigated. The periods during which the larvae received the iron-deficient diet are given in Table IV; in those cases where the iron-deficient leaves were not supplied until the later stages of development the initial diet consisted of complete-nutrient leaves. As in experiments 1 and 2, control groups of larvae received complete-nutrient leaves as food in each experiment.

Experiment 3.—This experiment was carried out in two parts and was designed to compare the effects of supplying the iron-deficient diet from the time of hatching (—Fe group A) and from the sixth day (—Fe group B). In each case a comparable group of larvae received complete-nutrient leaves throughout the period of the experiment.

(a) *Group A. Diet supplied from time of hatching* (fig. 4).—The control larvae had mean weights which were significantly higher than those of the iron-deficient group on each of the days when weighings were made. The relative growth rates, however, were almost identical ($b = 0.210$ in each case).

Larval development was not studied after the ninth day.

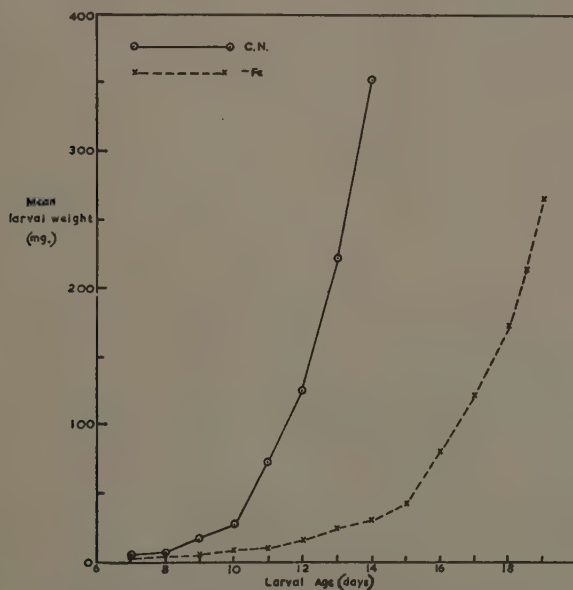


Fig. 5.—Growth curves for period day 6 to completion of larval life on diets of complete-nutrient and iron-deficient leaves, respectively, the larvae having been reared on complete-nutrient leaves from time of hatching to day 5. (Expt. 3b.)

(b) *Group B. Diet supplied from day 6* (fig. 5).—Once again, the mean weights of the iron-deficient group were significantly lower than those of the control group, and in this case the relative growth rate of the deficient group was also significantly lower than that of the control group (see fig. 6). In the deficient group, pupation was delayed by 5.9 ± 0.6 days.

It was clearly shown by this experiment that the iron-deficient diet caused a marked reduction in larval weights whether supplied from the time of hatching

or only from the sixth day. However, the relative growth rate was not significantly reduced by the diet in the first case, whereas in the second it was, and this suggested that an initial severe check to growth (as evidenced by the lower mean weight on the sixth day) had been followed by some degree of recovery in the group given the deficient leaves from the start of larval life, whereas the group first receiving this diet on the sixth day were unable to recover to the same extent.

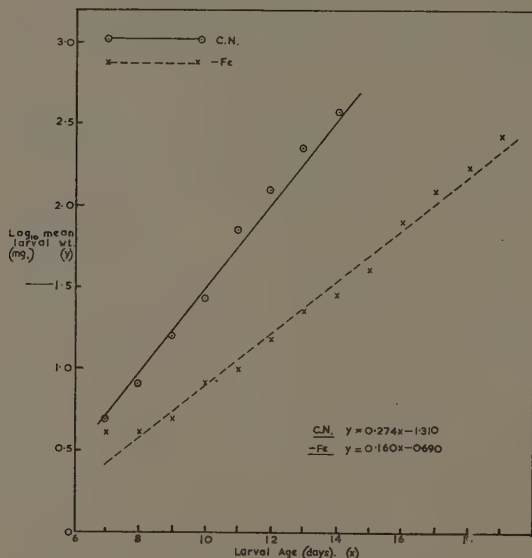


Fig. 6.—Comparison of relative growth rates for period day 6 to completion of larval life on diets of complete-nutrient and iron-deficient leaves, respectively, the larvae having been reared on complete-nutrient leaves from time of hatching to day 5. (Expt. 8b.)

Experiment 4 (figs. 7 & 8).—In order to verify the hypothesis formulated above, the experiments were repeated, the three groups of larvae, however, being studied simultaneously. One group received complete-nutrient leaves throughout the period of the experiment (C.N.), a second group received iron-deficient leaves for the same period (-Fe group A), and the third group had a change of diet on the sixth day of larval life from complete-nutrient to iron-deficient leaves (-Fe group B).

The mean larval weights of each group from the sixth to the 13th day of larval life are shown in fig. 7. Analyses of variance carried out on the figures for the seventh, ninth and eleventh days proved that the differences in mean weight between each group were significant (C.N. > group B > group A). Larval mortality from day 0 to day 5 was significantly higher ($p < 0.001$) in the group (A) receiving iron-deficient leaves during this period than in those groups (C.N. and group B) receiving complete-nutrient leaves. This latter result is in agreement with the high mortality of young larvae fed on nitrogen-deficient leaves in experiment 2.

Although no weighings were made after the 13th day, it can be stated that in both the iron-deficient groups pupation was delayed, since all the larvae in these two groups were still feeding on the 13th day, while all those in the complete-nutrient group had reached the pre-pupal stage on the 12th day.

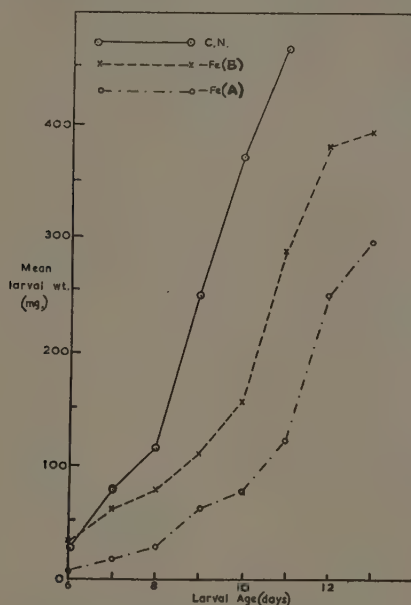


Fig. 7.—Growth curves for period day 6 to day 13 on diets of complete-nutrient and iron-deficient leaves, respectively, the larvae having been reared on complete-nutrient (curves C.N. and -Fe(B)) or iron-deficient (curve -Fe(A)) leaves from time of hatching to day 5. (Expt. 4.)

The relative growth rates (see fig. 8) were significantly different from one another (C.N. > group A > group B). Since group B had a significantly lower relative growth rate ($p < 0.01$) than group A, the result of the previous experiment is confirmed, *i.e.*, after the initial check caused by the deficient diet the relative rate of growth of group A increased until it exceeded that of group B.

The rates of growth of larvae in experiments 3a, 3b and 4 varied, due either to different environmental conditions (see Table I) or to uncontrolled factors, such as slight differences in the food supplied. Relative growth rates of groups of

TABLE V.

Ratios of regression coefficients of \log_{10} weight on time in experiments 3 & 4.

		Expt. 3	Expt. 4
Group A (-Fe diet from hatching) / Control	1.00	0.75
Group B (-Fe diet from sixth day) / Control	0.58	0.54

larvae in the different experiments cannot, therefore, be directly compared. However, within any one experiment the ratios of the relative growth rates, (*i.e.*, regression coefficients of \log_{10} weight on time) of iron-deficient to the control groups may be calculated. The ratios for experiments 3 & 4 are given in Table V.

It can be seen that the ratios for Group B are closely similar in the two experiments. Although there was less close agreement between the ratios of the

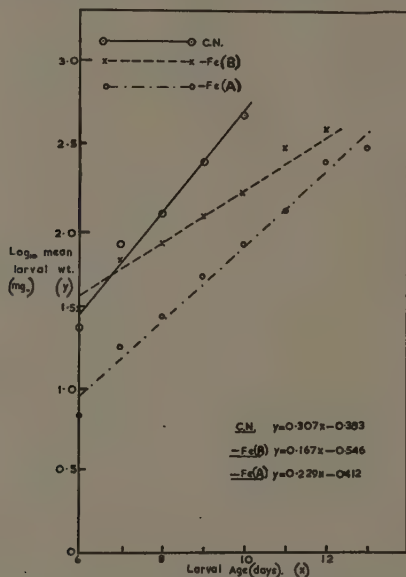


Fig. 8.—Comparison of relative growth rates from day 6 to day 13 on diets of complete-nutrient and iron-deficient leaves, respectively, the larvae having been reared on complete-nutrient (curves C.N. & -Fe(B)) or iron-deficient (curve -Fe(A)) leaves from time of hatching to day 5. (Expt. 4.)

two A groups, in both experiments the value of the ratio for the A group was greater than for the B group. This accords with the suggestion that the check to growth rate caused by the iron-deficient diet was overcome to a greater extent when the diet was given at the *beginning* of larval life than when it was first given after six days on a normal diet.

Experiment 5 (fig. 9).—The period on the diet of iron-deficient leaves was here limited to the last two larval instars. Mean weights are shown graphically in fig. 9, the difference between iron-deficient and control groups being significant on each day when weighings were made. As in previous experiments, the date of pupation was significantly delayed by the diet of deficient leaves and the relative growth rate was significantly reduced as compared with the control group (see fig. 10).

The ratio of the regression coefficient of the iron-deficient group to that of the control was 0.78, which was greater than the values given in Table V for the ratios of Group B to the controls in experiments 3 & 4. Although undue weight cannot be attached to this comparison, it suggested that the effect on the relative growth rate was less severe when the iron-deficient diet was supplied only during the last two larval instars than when supplied from the sixth day.

Pupal weights.—In experiments 3b and 5, the weights of the pupae produced were compared with those of the control groups. In the first case the mean difference between deficient and control groups was 69.0 ± 19.0 mg., and in the second the mean difference was 64.0 ± 9.1 mg., in both cases the iron-deficient group being significantly lighter ($p > 0.01$).

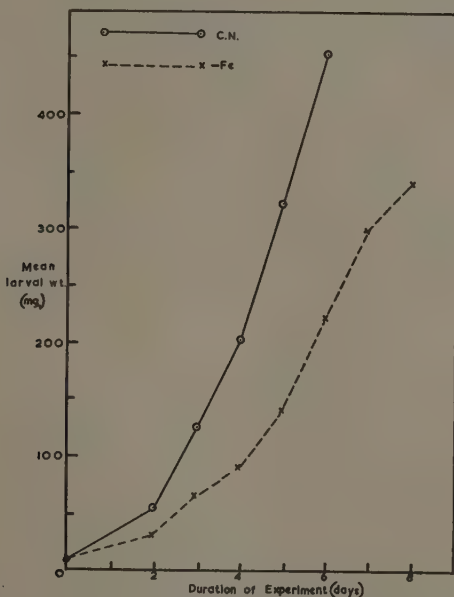


Fig. 9.—Growth curves for last two larval instars on diets of complete-nutrient and iron-deficient leaves, respectively, the larvae having been reared from time of hatching on complete-nutrient leaves. (Expt. 5.)

Supplements to iron-deficient diets.

A few preliminary tests were made on the effects of spraying iron-deficient leaves with aqueous solutions of various nutrients, whose concentration in the leaves could have been affected by the deficiency. A similar technique has also been used by Grison (1948), who supplied lecithin to potato leaves on which Colorado beetles, *Leptinotarsa decemlineata* (Say), were feeding.

In the present instance preliminary trials were made with sprays providing additional iron (ferric chloride: 0.4%), carbohydrates (mixtures of sucrose and glucose: 2.5–10%), protein (egg-albumin: 0.5–2.5%) and vitamin B (yeast suspension). In all cases the treated leaves were eaten by the larvae in apparently normal amounts, but no markedly beneficial results were noted as compared with the untreated leaves. The range of concentrations used was, however, limited, and further tests on these lines need to be made.

Discussion.

It has been demonstrated that diets of leaves showing symptoms of a deficiency of N, P, K or Fe had a detrimental effect on larval development, and in the case of Fe this was true for the three stages of development studied. This effect was most clearly reflected in the lower weights of the larvae after a short

time on the diet, as compared with those of the control larvae fed on complete-nutrient leaves. Increased mortality also resulted from these diets of deficient leaves in two experiments (-N and -Fe), occurring particularly in the earlier stages of larval growth.

The similarity between these results and those of Craig & Hoskins (1940) and of Kellogg & Bell (1903) is interesting. These authors reported slower larval growth, delayed pupation and smaller adults when the food supply of their

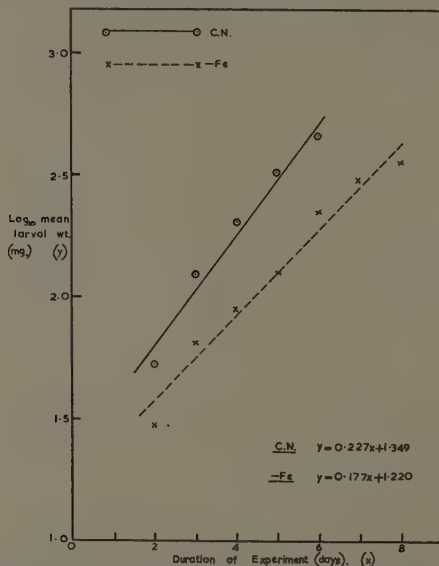


Fig. 10.—Comparison of relative growth rates for last two larval instars on diets of complete-nutrient and iron-deficient leaves, respectively. (Expt. 5.)

experimental insects was reduced. The similar effects observed in the present experiments would suggest that here also some form of starvation was responsible. However, if this is a correct assumption, it does not necessarily follow that the adverse effects were caused solely by a reduced food intake, since a shortage of one or more specific substances in the leaves might also have contributed, either wholly or in part. In view of the known effects of the various mineral deficiencies on the organic and inorganic constituents of plant tissues this seems most probable.

In addition to these general results, it has been demonstrated that, in spite of the greater reduction in weights of larvae fed on iron-deficient leaves from the time of hatching as compared with weights of larvae fed on this diet only after six days, the larvae of the first group were growing relatively faster after a few days had elapsed than the larvae of the same age in the second group. It is suggested that some form of adaptation to the unfavourable diet can occur, provided that the latter is supplied very early in larval life.

Summary.

When larvae of *Pieris brassicae* (L.) were fed on leaves of plants showing symptoms of deficiency of nitrogen, phosphorus, potassium or iron, some or all of the following effects were recorded in each experiment:—

Reduction of larval weight (deficiency of (1) N, P, K throughout larval life, (2) Fe from time of hatching, from sixth day and for the last two larval instars).

Reduction of relative growth rate (deficiency of (1) N throughout larval life, (2) Fe from time of hatching, from sixth day and for last two larval instars).

Increased larval mortality (deficiency of N, Fe from time of hatching).

Delayed pupation (deficiency of (1) N, P, K throughout larval life, (2) Fe from time of hatching, from sixth day and for the last two larval instars).

The more detailed experiments with iron-deficient diet showed that similar effects were produced at whatever stage of larval development it was first supplied.

Larvae fed on iron-deficient leaves from the time of hatching appeared to have received a severe initial check to growth, but this was followed by some degree of recovery so that they showed a higher relative growth rate, a few days later, than larvae given the same diet six days after hatching.

Preliminary trials were made on the effects of the addition to diets of iron-deficient leaves of various nutrient substances sprayed on to them. No markedly beneficial results were noted.

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DURATION OF THE AQUATIC STAGES OF *POVILLA ADUSTA* NAVÁS (EPHEMEROPTERA: POLYMITARCIDAE).

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The mayfly, *Povilla adusta* Navás, is widespread in Africa. The tubicolous larva (fig. 1) lives over a wide variety of substrata in rivers, lakes and ponds, and is a filter-feeder (Hartland-Rowe, 1953); its food consists of microscopic algae (Kimmins, 1949; Hartland-Rowe, personal communication, 1956). This insect is well known to boat-builders in East Africa because the wood-boring

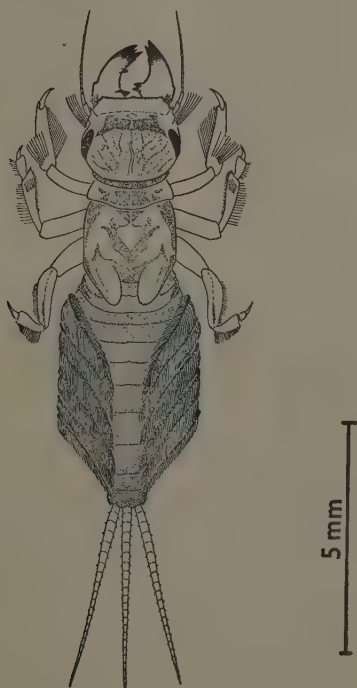


Fig. 1.—*Povilla adusta* Navás. Half-grown larva, about 6 weeks after hatching from the egg.

activities of its larvae can cause serious damage to structures beneath the water-line. In Lake Victoria, and doubtless elsewhere, both larvae and adults provide an important element in the food of several species of fish.

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A remarkable feature of the life-history is the swarming of adults which occurs every month shortly after full moon (Hartland-Rowe, 1955). It seems that most of the adults each month appear on a single night, smaller numbers emerging on two, or sometimes three, other nights just before or just after the main swarm. The regular appearance of the main swarm at Jinja ($33^{\circ}12\frac{1}{2}'\text{E.}$, $0^{\circ}25\frac{1}{2}'\text{N.}$) is shown in fig. 2. On the night of a swarm, adults emerge shortly after dusk. They

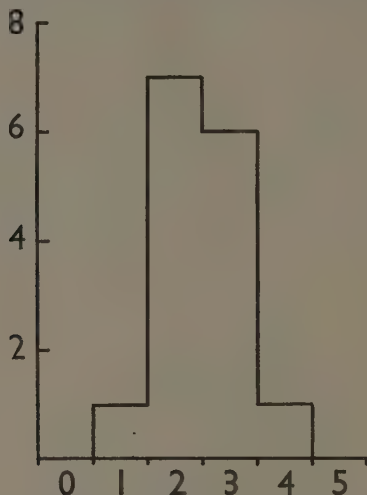


Fig. 2.—Times of main adult swarms at Jinja, in relation to full moon, from July 1954 to September 1955.

Abscissa: Days after full moon.

Ordinate: Frequency.

are rarely encountered at light before 1930 hr. (Local Mean Time) or after 2130 hr., and they only live for about an hour (Hartland-Rowe, 1955). The periodicity of emergence makes it possible to estimate growth-rate by the analysis of larval samples.

Methods.

Since larvae frequently inhabit holes in rock and wood, it is very difficult to collect them by conventional methods. It was therefore thought that satisfactory samples might be obtained from the stomachs of the fish, *Mormyrus kannume*

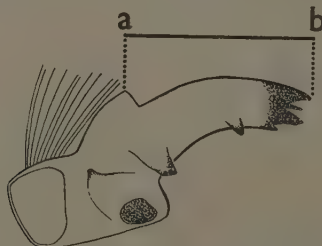


Fig. 3.—Left larval mandible in dorsal view, showing distance measured (ab).

Forsk., which feeds heavily on *P. adusta* larvae in certain habitats. Accordingly, larval samples were examined from the stomachs of fishes caught at regular intervals over appropriate feeding-grounds. A similar technique was used by Macdonald (1956) to determine the life-histories of certain CHIRONOMIDAE and CULICIDAE in Lake Victoria.

It would be unreasonable to claim that samples derived in this manner are representative of the whole larval population. *A priori*, there are two circumstances which may be expected to reduce the chances of fishes eating either particularly small or particularly large larvae. In the first place, fishes do not eat *P. adusta* larvae of below a certain size (ca. 3 mm. length). We may therefore expect the "availability" of larvae to show a progressive increase above this size. On the other hand, mortality during larval life will have the opposite effect, making large larvae less available than small ones. The resultant of these conflicting factors should be that fishes will tend to feed mainly on larvae of an intermediate size. This tendency for fishes to feed on medium-sized larvae was found to be a frequent feature of larval samples. Therefore, for purposes of analysis, especial attention has been given to length-frequency modes amongst smaller larvae, since these seem to result from excessive abundance of a particular age-group, rather than from selective feeding on the part of the fishes.

The fishes were caught in gill-nets, set in the evening and lifted in the morning, and situated about 50 yards from the shore of Lake Victoria, near Jinja, Uganda. The nets were set over a bottom of gravel, rock and sand, on which grew submerged plants; the depth was about 20 feet. In this habitat, *P. adusta* larvae provided the second most important item in the food of *M. kannume*, occurring

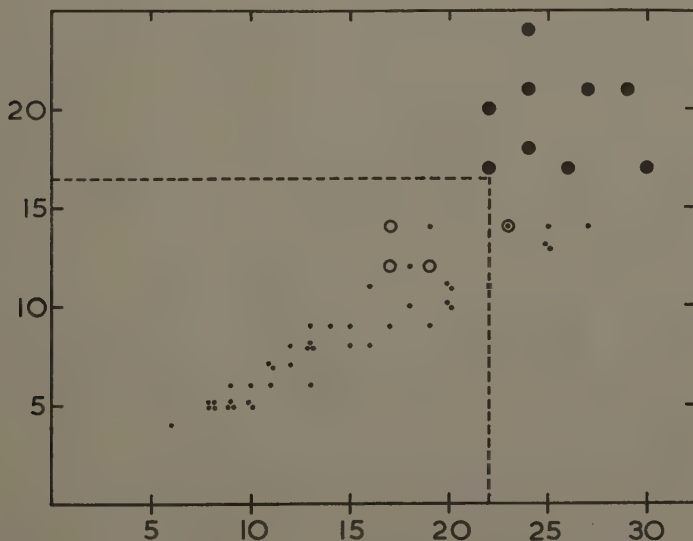


Fig. 4.—Relationship between mandible size and body length. Small dots: unsexed larvae. Empty circles: male larvae undergoing metamorphosis. Full circles: female larvae undergoing metamorphosis. The dotted line indicates the average size at which metamorphosis takes place in male and female larvae.

Abcissa: Mandible size, in eye-piece units.

Ordinate: Body length, in mm.

in 49.6 per cent. of all fishes that contained food, and occupying more than half the volume of food in 15 per cent.

After the total length of a fish had been measured, its stomach contents were examined and all head-capsules of *P. adusta* larvae removed. These were placed flat on a microscope slide and the left mandibles were measured from a dorsal aspect, using a micrometer eye-piece scale. Only mandibles attached to heads were recorded. The distance measured is shown in fig. 3, the eye-piece units being such that $17\frac{1}{2}$ were equivalent to 1 mm. The relationship between mandible size and body length (excluding the anal cerci) is shown in fig. 4.

There were indications that the size of larvae eaten depended to some extent on the size of fish (see fig. 5) and therefore measurements of *P. adusta* for each fish were recorded separately.

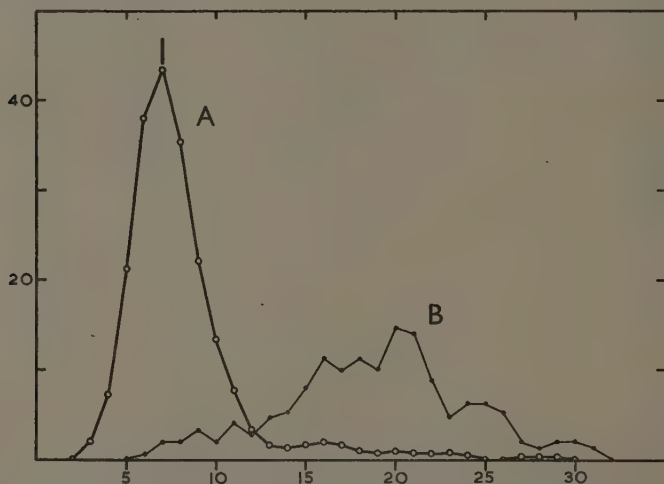


Fig. 5.—Length-frequency distributions of larvae eaten on 13th September 1954 by two fishes, 20 cm. (A) and 31 cm. (B) total length. This date was 27 days after the last swarm. A thick vertical line indicates the mode of sample A.

Abscissa: Mandible size, in eye-piece units.

Ordinate: Frequency, smoothed by a moving average of 3.

The onset of metamorphosis is usually associated with changes in the pigmentation of the wing-sheaths (Hartland-Rowe, personal communication, 1956), and this fact was used to estimate the average size at which larvae can metamorphose. It was found that males metamorphose at a smaller size (average mandible size: 19 units) than females (25.3 units). In the present study, where most of the material consisted of detached heads, it was not possible to distinguish the sexes. It has therefore been assumed that they were represented approximately equally in samples, and the mean of the male and female average mandible sizes (*ca.* 22 units) has been used to determine when a larval population could begin to metamorphose.

Results.

Between 21st July and 21st October 1954, 4,403 larvae from 44 fishes were measured. The total size-range of these fishes was 19–43 cm., and was determined by the mesh of the gill-nets used for capture. In the majority of

cases, however, the length-frequency modes amongst smaller larvae were not sufficiently well-defined to be useful. In general, this was due either to the sample being too small, or else to the fish having fed mainly on large larvae; the latter circumstance was common in the case of fishes more than 30 cm. long. Accordingly eight samples, comprising a total of 993 larvae, were selected for consideration. These samples stood apart from the rest in having well-defined

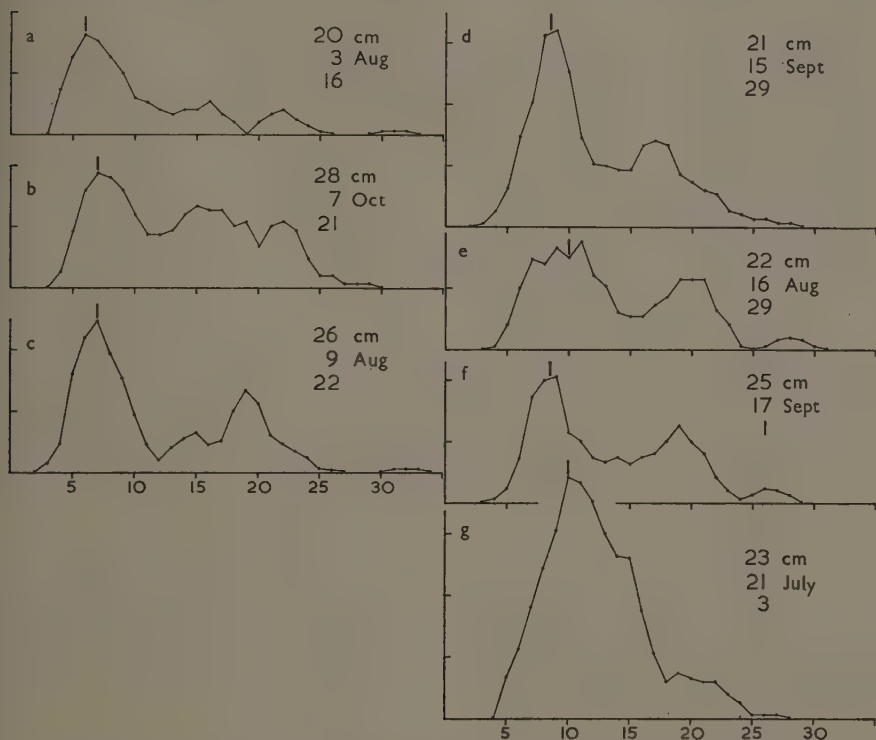


Fig. 6.—Length-frequency distributions of larval samples from fish stomachs. Thick vertical lines indicate the modes used in fig. 7 to estimate larval growth-rate. For each sample is given the total length of the fish, the date upon which it fed, and the number of days that had elapsed since the last swarm.

Abcissa: Mandible size, in eye-piece units.

Ordinate: Frequency, in units of 5, smoothed by a moving average of 3.

modes and in coming from fishes between 20 and 28 cm. long. The length-frequency distributions of these samples form the subject of figs. 5 and 6; in fig. 7 their smaller modes are shown in the context of the life-history.

The periodicity of adult swarms makes it clear that the duration of the aquatic stages must be a whole number of lunar months. But before the length of larval life can be inferred from larval samples, it is necessary to know how long the eggs take to hatch.

Duration of the egg stage was determined by keeping egg-masses in the laboratory. The times elapsing before hatching occurred are given in Table I. Egg-masses were obtained from gill-nets, in which they sometimes became

entangled, or from adult females that had been attracted to light. Only in the latter case was it known exactly when eggs were laid; the date of laying of eggs found on gill-nets was deduced from the time of the previous main swarm.

The results in Table I refer to eggs kept in lake water, since it was found that hatching was delayed in tap water. The temperature at which eggs were kept

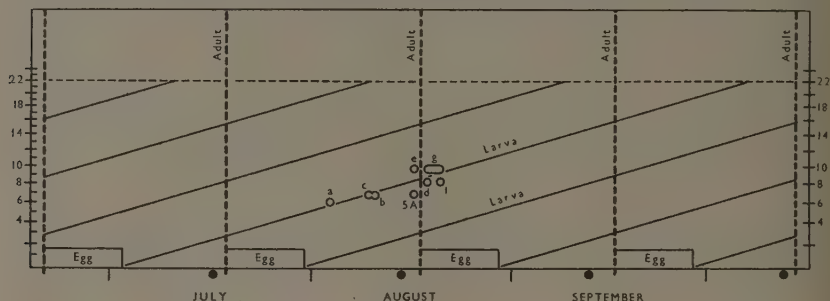


Fig. 7.—Diagram of life-history. Dates of the main swarms in July, August and September 1954 are indicated by thick vertical broken lines, and the times of full moon by filled circles on the abscissa. The egg stage and the modes of larval samples shown in figs. 5 and 6 have been inserted. Although derived from different months, these modes have been placed in the appropriate positions for August only. This has been done by assuming that all the larvae measured came from eggs laid in the June swarm. Thus, maximum and minimum values are indicated for the July mode (g), since the date of the May swarm (when the eggs of this age-group would supposedly have been laid) is unknown. The upper horizontal broken line shows the average size at which metamorphosis occurs. The lines representing larval growth-rate have been fitted by eye. It can be seen that a generation is completed in 4 or 5 lunar months.

Abcissa: Months in 1954.

Ordinate: Mandible size of larvae (log. scale).

(ca. 20°C.) was slightly below that which they would experience on the bottom of Lake Victoria, either in deep or shallow water. (Bottom temperatures at 19 metres, near Buvuma Island, over the period 1951–53 varied between 23.8 and 26.7°C. (Fish, personal communication, 1954).) Thus, if egg development has a

TABLE I.

Duration of egg development.

Date of main swarm	Date of collection	Date of first hatching	Maximum duration (days)
1954 17 Aug.	19 Aug.	6 Sept.	18
16 Sept.	17 Sept.	4 Oct.*	17
1955 8 Apr.	8 Apr.†	26 Apr.*	18
9 May	11 May	25 May*	16
9 May	20 May	21 May	12
7 June	11 June	22 June	15

* On or before date given.

† Exact date of laying; oviposition observed.

positive temperature coefficient, its duration in nature will be somewhat shorter than it is in the laboratory. In support of this, we may note the rapid hatching of eggs obtained on 20th May 1954 (Table I). These were presumed to have been laid on the night of the main swarm, and to have hatched after only 12 days, 11 of which would have been spent in the lake. Another batch of eggs, laid in July 1955 and allowed to remain in the lake throughout the incubation period, began hatching after only 11 days (Hartland-Rowe, personal communication, 1956). It is reasonable to assume that about 12 days is the usual duration for egg development in nature at this latitude.

Returning to fig. 7 with this in mind, we can see that the best fit (by inspection) for growth-rate involves most of an age-group reaching the average size for metamorphosis about eight days before a swarm.* It is not known whether this period is sufficient to allow all larvae to metamorphose, although it is probable that some are able to do so, since ripe eggs have not been discerned in female larvae earlier than three days before a swarm. There can be little doubt, however, that those unable to complete metamorphosis in this time would emerge the following month. Thus, a single generation must be completed in either four or five lunar months, a finding which necessitates a revision of Kimmins' earlier opinion (1949) that more than one year is required for larval development. Further work is needed to determine whether male larvae (on account of their smaller size) reach maturity more quickly than female.

The possibility that larvae could reach a modal size of six units in two days (thus reducing the estimated duration by one month) has been shown to be unreasonable by rearing larvae in the laboratory. Lack of suitable food could not have been responsible for the difference observed, since larvae retain the vitelline cells in the mid-gut for several days after leaving the egg, and at least until the 2nd instar (Hartland-Rowe, personal communication, 1956).

Some recent observations (Hartland-Rowe, personal communication, 1956) lend support to this interpretation of the life-history. Larvae from a single egg-mass, kept in a cage on the lake bottom, reached a modal mandible size of 11.8 units 51 days after hatching, whereas the comparable value derived from fig. 7 is 59 days.

Discussion.

These findings indicate that, as a rule, three generations are completed in 12 to 15 months, and that four or five age-groups usually co-exist in a population. For slightly less than half of the time (i.e., during the first 12 days of each lunar month) one of these age-groups is present as eggs; for the rest of the time, all age-groups exist as larvae.

This periodic change in the composition of the population affects the feeding habits of several species of fish. *M. kannume*, the principal predator of larval *P. adusta* in certain habitats, feeds on a greater volume of larvae at full moon than at other times. Also, the number of larvae found in stomachs shows an abrupt decrease shortly after a swarm has taken place. Probably other species of MORMYRIDAE are affected similarly. Despite these fluctuations, however, the existence of four temporally-separated age-groups means that larvae of a wide size-range will always be available as food for fishes.

The lunar rhythm of *P. adusta* has a still greater effect on surface-feeders or generalised predators. Thus, at the time of a swarm, it is common for such

* Only the smallest size-mode of each sample has been included in fig. 7. As mentioned above, the larger modes are thought to be less reliable, due to selective feeding on the part of the fishes. If, however, the larger modes are plotted in fig. 7, they fall very close to the growth-lines of the older age-groups, and thus lend support to the interpretation based on the smaller modes. Actually, values for the second largest modes fall slightly above the fitted growth-line, implying that larvae may in fact reach the average size for metamorphosis rather sooner than eight days before a swarm.

fishes as *Alestes nurse* Rupp., *Barbus altianalis* Blgr., *Bagrus docmac* Forsk., and *Clarias mossambicus* Peters to feed exclusively on emerging individuals.

P. adusta provides an extreme example of an insect in which a short adult life is associated with efficient synchronisation of emergence. The biological significance of this synchronisation may not be connected entirely with the short life of the adult. Some relatively short-lived insects seem to show a more or less continuous emergence near the equator (see Corbet & Tjønneland, 1956). It may be that *P. adusta* exists at too low a density for continuous emergence to be practicable from a reproductive point of view. Although dense masses of adults are encountered around lights on swarm nights, the nightly numbers would be small were these adults to be distributed evenly throughout a month.

In temperate regions, the seasonal change in temperature is thought to play an important part in determining the need for synchronised reproduction. In a tropical environment, where conditions may often be suitable all the year, it may well be that low density is closely associated with the need for synchronisation of the reproductive stage. Thus, we might expect that only exceptionally abundant species would breed continuously throughout the year.

Summary.

The duration of the aquatic stages of the mayfly, *Povilla adusta* Navás, in Lake Victoria, Uganda, has been determined by analysing larval samples from the stomachs of an insectivorous fish, *Mormyrus kannume* Forsk., and by culturing eggs *in vitro*. Emergence shows a well-defined lunar rhythm and, since adults live for only about one hour, duration of the aquatic stages must be an integral number of lunar months. Results indicate that a generation is usually completed in four or five months. Approximately the first two weeks of this period are spent in the egg.

The periodicity of reproduction imposes a feeding rhythm on certain species of fish in Lake Victoria. Some biological implications of the need for synchronisation are briefly discussed.

Acknowledgements.

It is a pleasure to express my gratitude to Mr. Richard Hartland-Rowe of Makerere College, Kampala for his advice and criticism, and for allowing me to quote his unpublished observations.

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THE GENUS *MUSA* LINN. AND ITS RÔLE IN THE BREEDING OF *Aedes (Stegomyia) simpsoni* (THEO.) ON THE KENYA COAST.

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During yellow-fever control measures in ports along the Kenya littoral from 1939 to 1952, banana plants were an important source of two species of mosquito, *Aedes (Stegomyia) simpsoni* (Theo.) and *A. (S.) aegypti* (L.) (Teesdale, 1941) and no satisfactory method could be found of preventing breeding in them. Dusting with pyrethrum or DDT powder had proved impracticable owing to wide distribution and great numbers, and since the fruit is of considerable value as a food for the African population, the plants could not be eliminated by wholesale destruction.

Haddow's work in Bwamba County, Uganda (Haddow, 1948) prompted the writer to examine the potentialities of different varieties in the hope that some might prove to be unsuitable as breeding sites of these mosquitos. If such were found, then it seemed that control might be materially advanced by restricting propagation to these kinds only.

The Varieties examined.

Between 1946 and 1950, attempts were made to collect information regarding the different varieties that are grown on the coast. This proved by no means an easy task as some of the plants often had different local names and the fruit was used for various purposes by the different local tribes. After personal inspection of a very large number of plants it was possible eventually to draw up Table I, which probably includes all the common forms.

Local names are given, as all forms, with one exception, are varieties of the one named species *M. sapientum* L., the exception, that known locally as "Mkono wa Tembo", is a plantain (*M. paradisiaca* L.).

Height, length and shape of petioles and colours vary, so that no more than a very general indication of these points can be given.

There is some doubt as to whether Kiguruwe and Ya Kike are, in fact, the same plant; but as they appeared to be indistinguishable they are considered as synonymous in this paper. The differences between Kisukari and Kipukusa are also slight, and it is possible that in some instances both varieties are recorded as one. Mlali and Mshale are possibly the same and they together with Mrau may well be local names for one of those already included. Since this point could not be confirmed they have been listed separately. Mboma is not strictly a coastal species but will probably be introduced in the near future.

Localities and Methods.

Examinations of plants were undertaken in two well-separated parts of the province. The first, at Kwale, which is situated at about 1,200 ft. above sea level in the Shimba Hills and about 20 miles south of Mombasa, was particularly suitable as all the listed forms were represented in extensive plantations of the Forest Department near the forest edge. The second was around a small village called Ganda about 100 miles north of Kwale and almost at sea level. Here all the listed forms except Mboma were also represented, but were less easily accessible.

Routine observations were made and records kept at Kwale from June 1950 to October 1951 and at Ganda from November 1951 to January 1952.

At Kwale, about 12 plants of each variety were examined daily (not the same plants); there were considerably fewer plants of Kibungala and Mkono wa Tembo than of the other forms. A long glass pipette was used for testing all accessible

TABLE I.

Plant	Height	Petioles		Midribs	Fruit		Remarks	
Variety*		Length	Gutters		Shape	Colour	Use	
Ya Kiume ..	Tallish 12-20 ft.	About one ft.	Almost closed	Tinged with pink	Curved, narrow, round	Green	Sweet ; eating	Stems pinkish yellow
Kiguruwe (Mdundasi) (Ya Kilke) (Kinu) (Malindi)	Short 6-10 ft.	Short 6-8 in.	Open	Green, some- times tinged with pale purple	Slightly curved; angular in cross- section	Yellowish green, mottled with brown	Sweet ; eating	Petioles green, with purple tinge on edges of gutter
Kibungala (Njoho) (Muhabasi) (Mchusi wa Kamba)	Tall 14-20 ft.	About 2-3 ft.	Open ; edges everted	Purple	Stout	Red	Cooking and eating	Not widely grown
Bokoboko (Mabulu)	Tall 15-18 ft.	3-3½ ft.	Almost closed	Pale green	Angular in cross- section	Yellow- green	Cooking	
Kisukari ..	12-16 ft.	2-3 ft.	Narrow	Green with narrow purple fringe to edges	Short, stout	Yellow	Sweet ; eating	
Kipukusa ..	6-10 ft.	1 ft.	Narrow	Pale purple	Small, curved	Yellow	Sweet ; eating	
Mkono wa Tembo	15-20 ft.	2-3 ft.	Open	Green	Large, curved, angular	Green	Brewing beer ; cooking	A plantain
Mboma	10-15 ft.	1½-2 ft.	Open ; edges dark purple	Pale yellow	Small, stout ?	Ripe fruit not seen	Eating and cooking	Brownish purple at base of petiole ; stems blotchy

Named but not seen: Mlali, Mshale, Mrau.

* Names in brackets are alternatives.

axils for water and mosquito larvae. If water was present, its volume was measured on the spot, and larvae were collected for identification. Similar methods were employed at Ganda except that a variable number of plants of each form was examined daily, and, whereas at Kwale the water was not returned to the axils, at Ganda it was.

It was found that *A. simpsoni* was virtually the only species present, and, when larvae are mentioned, those of *A. simpsoni* are intended unless otherwise stated.

The findings in the two localities are summarised in Tables II and III.

It soon became evident at Kwale (Table II) that no mosquitos were breeding in Kibungala, the least common variety, but as the plants were fairly young and in a more exposed position than the rest, it was decided to look for others elsewhere. A few were found at Vanga, a small town on the coast about 60 miles south of Kwale, and examinations were made once weekly during September and December 1950 and in June 1951. Larvae of *A. simpsoni* were found on one occasion.

At Ganda, however (Table III), there were more plants and searches there showed that *A. simpsoni* used them for breeding sites; but it seems clear that breeding does not occur in them to the same extent as in other plants although they have

TABLE II.

Summary of data collected from *Musa* species at Kwale, June 1950 to January 1952.

Variety	Plants ex- amined (no.)	Axils (no.)	Axils with water (no.)	Axils with water (%)	Average content per axil (cc.)	Axils with larvae (no.)	Water- bearing axils with larvae (%)
Mkono wa Tembo ..	215	2905	1236	42.5	3.9	131	10.6
Ya Kiume	574	5828	2393	41.0	5.2	197	8.2
Bokoboko	570	8329	2802	33.6	4.1	154	5.5
Mboma	563	5733	1598	27.9	4.5	56	3.5
Kiguruwe	530	6280	1487	27.3	3.1	35	2.4
Kisukari	556	6856	1777	25.9	3.5	82	4.6
Kibungala (Kwale) ..	278	2689	545	20.3	3.1	0	0.0
Kibungala (Vanga) ..	?	589	174	29.5	?	1	0.6

The number of larvae per axil was not counted at Kwale.

It is possible that some Kipukusa are included with Kisukari as the plants are similar.

no fewer water-bearing axils. (The high percentage of axils with water in this variety at Ganda during November is not comparable with the others, which were measured in the drier months of December and January, and for this reason have been listed separately).

It seems likely, therefore, that if propagation of bananas were restricted to this variety alone, little would be achieved in control since *A. simpsoni*, deprived of other plants for egg-laying, would probably use Kibungala to a far greater extent than at present.

Water-bearing Capacity and Mosquito Productivity.

Before any deductions can be drawn from the data given in Tables II and III, the criteria for measuring the capacity of a plant as a producer of larvae must first be considered and this seems to involve a number of factors.

In the first place the greater producers are the plants whose axils are capable of holding water and not the plants with the most leaf axils. Also, the percentage of water-bearing axils varies considerably in the different varieties; thus at both Kwale and Ganda it was highest in Mkono wa Tembo and Ya Kiume and lowest in Kisukari and Kibungala.

Secondly, the number of water-bearing axils with larvae must be taken into consideration for although, generally speaking, the more axils holding water the higher the number likely to contain larvae, this may not always be the case; for example, Mkono wa Tembo at Ganda, though having the highest proportion of water-bearing axils, had by no means the highest percentage with larvae.

Thirdly, the volume of water contained in any axil might be expected to bear a relation to the number of larvae it could support. This, however, appeared to be of little consequence in the present study since often a very small volume would contain more larvae than a large one, and both water content and larval counts seemed to vary greatly, as will be shown later.

TABLE III.

Summary of data collected from *Musa* species at Ganda, November 1951 to January 1952.

Variety	Plants ex- amined (no.)	Axils (no.)	Axils with water (no.)	Axils with water (%)	Average content per axil (cc.)	Axils with larvae (no.)	Total larvae from all axils	Water- bearing axils with larvae (%)
Bokoboko ..	391	3443	196	5.7	4.2	38	95	19.4
*Kiguruwe ..	314	3052	171	5.6	5.0	26	46	15.2
Ya Kiume ..	358	3382	214	6.3	3.7	31	87	14.5
Kipukusa ..	494	4141	195	4.7	4.4	29	59	14.8
Mkono wa Tembo	281	2645	179	6.8	3.2	20	43	11.2
Kisukari ..	201	1708	45	2.6	4.3	0	0	0.0
Kibungala (Nov.)	?	2378	323	13.6	2.2	12	?	3.7
Kibungala (Dec./Jan.)	219	1830	71	3.9	2.9	4	6	5.6

* Known as Ya Kike at Ganda.

There is some likelihood that an error occurred in the records of Kipukusa and Kisukari as the plants are very similar.

No explanation is offered for the absence of larvae in Kisukari.

Thus, it seems that in assessing the potentialities of any variety as a producer of larvae of *A. simpsoni* the criteria must be the proportion of axils which hold water and the proportion of these which support larvae.

A combination of these two data appear to give the same results at both Ganda and Kwale.

Zoning of the Plants.

At Kwale, each plant was roughly divided into three zones as follows:—

Top zone: Petioles making an angle of less than 45° with the stem.

Middle zone: Petioles making an angle greater than 45° and less than 90° with the stem.

Lower zone: Petioles making an angle of 90° and over with the stem.

In the top zone, water was found in all varieties at least once every month over the whole period of 20 months; in the middle zone, only Bokoboko, Mkono wa Tembo and Kisukari had water in their axils every month, the others being dry in February and March or March only; in the lower zone, all varieties were dry in February and March.

These findings differ from those recorded in Mombasa, where in 1941 the writer was unable to find any water present in the axils of a large number of banana plants examined after 43 days of complete drought (Teesdale, 1941). At that time, however, special attention was not paid to what is here referred to as the top zone, but only a general sample was taken that included all three zones. The presence of water found in axils at Kwale after 55 days without rain might be attributable to its higher altitude, where condensation at night is probably greater and evaporation during the day less than at sea level.

TABLE IV.
Average water content of leaf axils of *Musa* varieties at Kwale, May 1950 to January 1952.

Variety	Axils								
	Top Zone			Middle Zone			Lower Zone		
	With water (no.)	Average content (cc.)	With larvae (%)	With water (no.)	Average content (cc.)	With larvae (%)	With water (no.)	Average content (cc.)	With larvae (%)
Bokoboko ..	1247	4.3	5.0	1148	3.6	5.3	407	4.3	7.6
Mkono wa Tembo	556	4.2	10.9	505	3.7	12.5	175	3.8	4.0
Kigurwe ..	692	3.2	3.3	605	2.8	1.8	190	3.2	0.5
Kibungala ..	241	3.6	0.0	225	2.6	0.0	79	3.0	0.0
Kisukari ..	886	3.5	3.5	654	3.2	5.4	237	3.7	6.8
Ya Kiume ..	979	5.7	8.8	1085	4.6	9.4	329	5.2	2.7
Mboma ..	826	4.4	4.2	569	3.9	3.0	203	5.1	1.9
Average		4.1			3.5			4.0	

The average water content per axil over the 20 months was, without exception, greater in the top and lower zones than the middle (Table IV). At Ganda there was some variation but records were kept there for three months only. The percentage with larvae, however, was generally highest in the middle zone in all varieties.

These conditions may be accounted for to some extent by leakage and evaporation, both processes being greater in the wider axils of the middle and lower zones because the petioles are not always in close contact with the stem, and the water surface is greater. That larvae were found more often in the middle zone might be explained if it could be shown that the female mosquito finds the wider axils of this zone more accessible than those of the top. It is also probable that eggs get carried from higher to lower axils when the water overflows after showers of rain.

At the base of the stems, where a space forms between old petioles and the trunk, larvae are also able to exist. This site was examined at Kwale at the same time as the higher axils and was found to contain comparatively large quantities of water (over 100 cc. in Bokoboko). This variety appears to harbour many more larvae at this point than do the others, as they were found there on 21 occasions, only once in Kisukari and Mboma and not at all in the others. It is difficult to suggest how larvae gain access to these sites, as the upper part and the edges of the old petiole are almost invariably closely adpressed to the stem. Eggs may get washed into them in some way, but it seems improbable that adults are able to emerge.

Species recorded from Leaf Axils of *Musa*.

The only species recorded at both Kwale and Ganda other than *A. simpsoni* were *Aedes metallicus* (Edw.) (twice in Bokoboko, Kisukari, Ya Kiume and

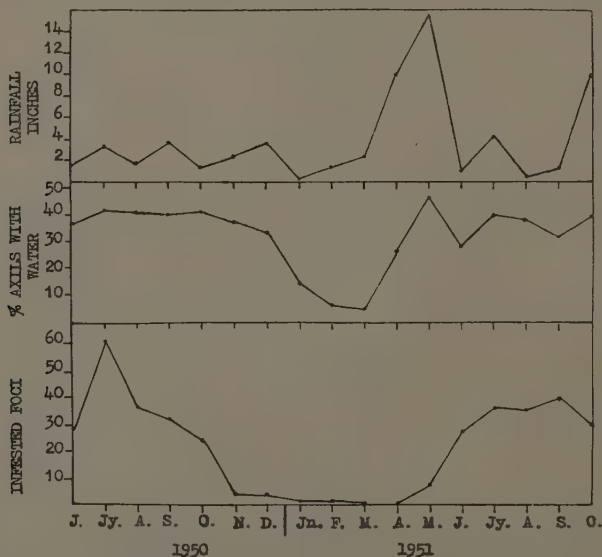


Fig. 1.—Seasonal distribution of *Aedes simpsoni* recorded in leaf axils of *Musa* varieties, Kwale, June 1950–October 1951.

Mboma); *Aedes aegypti* (once in Mkono wa Tembo) and *Eretmapodites chrysogaster* Grah. (once in Bokóboko with *A. simpsoni*).

Records from Mombasa from 1942 to 1944 show that banana plants there are one of the chief sources of *A. simpsoni*, though it was also recorded in tree holes, coconut-shells, snail-shells (*Achatina*), tins and bottles (van Someren, Teesdale & Furlong, 1955). The records from banana plants at Mombasa were:—

<i>Aedes</i> (<i>S.</i>) <i>simpsoni</i>	656 times
<i>Aedes</i> (<i>S.</i>) <i>aegypti</i>	78 „
<i>Aedes</i> (<i>S.</i>) <i>metallicus</i>	once
<i>Eretmapodites chrysogaster</i> group	41 times
<i>Eretmapodites quinquevittatus</i> Theo.	10 „
<i>Culex</i> (<i>Culiciomyia</i>) <i>nebulosus</i> Theo.	once
<i>Culex</i> (<i>Neoculex</i>) <i>horridus</i> Edw.	once

Seasonal Output of *A. simpsoni*.

A. simpsoni was by far the most common mosquito recorded from banana plants both at Ganda and Kwale and its seasonal prevalence is indicated in fig. 1. No significant difference was found in the monthly output of larvae from the several varieties examined. The curve is, therefore, derived from the number of times the mosquito was recorded, in all the varieties examined, over the period June 1950 to October 1951.

The curves delineating precipitation and percentage axils with water show a similar trend, but those for precipitation and the production of *A. simpsoni* do not. With the exception of the period of drought from January to March, neither the maximum nor minimum rainfall corresponded with the maximum or minimum number of foci. The following data make this clear.

Rainfall			Heavy			Light		
Month	Apr. 1951	May 1951	Oct. 1951	July 1950	Aug. 1951	Sept. 1951
Rain (in.)	10.5	15.61	10.09	3.31	0.56	1.38
No. of foci	..		0	8	29	61	34	41

These findings agree with earlier ones in Mombasa where heavy rain was usually associated with low larval output; but it is thought that no breeding was found in April because gravid females were scarce after the preceding dry period and many eggs already laid before then were washed out by the heavy rain. By May a gradual increase in the mosquito population was being reflected in the eight foci found during that month. Rainfall from then onwards was much less, but adequate, and larval output was maintained from July to September at an average monthly figure of 37 foci. Consequently, by October there was already a sufficient number of gravid females in existence to keep larval production at a fairly high level even when ten inches of rain fell and many eggs must have been washed away.

However, there are almost certainly other factors involved. Gillett (1955) has shown that the hatching response of *Aedes* eggs is governed to a considerable extent by a rapid but limited fall in temperature. It is not known whether this occurs in leaf axils of banana at the coast.

The highest monthly percentage of leaf axils with water was recorded in May and was 46. This figure probably expresses the average maximum number of axils capable of holding water in a banana plant.

Water Content of Axils.

The curve for the percentage of axils with water is almost certainly a true one, but it is only indirectly dependent upon precipitation, for heavy rain will not keep axils filled with water any more than will light showers so long as both are fairly continuous; but it is extremely difficult, if not impossible, to compare the capacity of different plant varieties to hold water. It was found that the content varied considerably from day to day and even from hour to hour. In order to assess this variation more precisely, five Bokoboko plants were chosen, each of which had one axil that contained both water and larvae. The water was measured daily at 7 a.m. and 6.30 p.m. and any larvae recovered were scrutinised and the instars

TABLE V.

Records taken over a 15-day period (1st to 15th December 1951) at Ganda, of water content and larvae in a leaf axil of a banana plant.

Rainfall (Malindi) (in.)	Day	7.00 a.m.			6.30 p.m.		
		Content (cc.)	Larvae	Instar	Content (cc.)	Larvae	Instar
0.00	1	7.2	1 2	IV II	3.8	1	IV
0.00	2	3.4	1	IV	1.0	1	IV
0.00	3	1.2	0	—	0.0	0	—
0.00	4	0.0	0	—	0.0	0	—
0.00	5	0.0	0	—	0.0	0	—
0.00	6	3.0	2 4	II I	0.0	0	—
0.00	7	3.0	1 2	II I	0.0	0	—
0.00	8	2.8	1 2	II I	1.0	2 3	III II
0.00	9	3.2	4	III	0.0	0	—
0.00	10	1.0	2	II	0.0	0	—
1.25	11	3.0	2 1	IV II	2.3	1	IV
0.68	12	3.0	1	IV	3.6	2 1	IV III
0.65	13	6.0	1 3	IV II	Tr.	1	IV
0.00	14	3.0	(1 pupa) 1 1	— IV II	2.4	(1 pupa) 1 1	— IV II
0.00	15	Tr.	(1 adult)	—	0.0	0	—

Meteorological data were not kept at Ganda, but a note was made of the days on which rain fell. Rain also occurred on the same days at Malindi (almost at sea level, about 4 miles from Ganda).

roughly estimated; they were then replaced with the water. Examinations were made over a 15-day period. As findings were similar in all five axils, those for one only are given in Table V.

These figures show that there is nearly always a loss of water during the daytime and that often it is apparently complete; by the following morning, however, it has sometimes been replenished. Such replenishment did not always coincide with rain and is probably due to condensation at night. Consequently it is clear that unless all varieties are examined at the same time each day (which was not done) a comparison of the average content becomes impossible and therefore the figures relating to this in Tables II and III are of doubtful value.

Even when there has been no measurable water present for some time, larvae are evidently able to survive in the film on the inside of the axils (Haddow, 1948) as they are often taken after it had been impossible to obtain water from the axils for some days. For example, at the 6 p.m. examinations as many as five successive days elapsed without water or larvae being recorded, but on the sixth evening third-instar larvae were found.

In collecting larvae therefore with a pipette it may often appear that none are present when in reality they cannot be captured because of insufficient water in the axils and consequently the output per plant, as Haddow has already indicated, is probably much greater than collections of larvae would lead to suppose.

These conditions, coupled with the finding of predacious *Eretmapodites* larvae in association with those of other species of mosquito, make an assessment of the number of larvae per water-bearing axil virtually impossible.

The greatly diminished water content in axils which occurs periodically also probably plays some part in the retarded growth of larvae previously noticed (Teesdale, 1941).

Conclusions.

It has been shown that, of the varieties of banana plants found on the Kenya coast, all those that were tested are capable of providing breeding sites for *Aedes simpsoni*, but that some kinds appear to be more suitable than others. A definition of such "suitability" is difficult because there appears to be no definite relationship between the number of water-bearing axils or their water content and the number of larvae present.

It is not considered that control of the mosquito would be improved by restriction of propagation to the less suitable plant varieties.

Although no larvae were found in March and April, it is possible that some breeding continues throughout the year since it is shown here that larvae exist in leaf axils even when the latter appear to be dry.

Summary.

The varieties of the banana plant (*Musa*) on the Kenya coast, with particular reference to *Aedes simpsoni* (Theo.), a species concerned with the transmission of yellow fever in East Africa, are described, and the species of mosquito larvae found in their leaf axils are recorded. *A. simpsoni* was virtually the only species present.

Details are given of the proportion of leaf axils that contain water and the percentage of these that produce larvae. Breeding was found to take place in all varieties, but to a much lesser extent in that known as Kibungala than in the others. Although there was often more water in the lower and upper axils than in the middle ones they produced less larvae individually than did the latter. Larvae were also found in old axils at the base of the stems in spite of the fact that these sites seldom appear to provide direct access for an ovipositing mosquito. Water was present throughout the year in the upper axils only.

It was found that the water content of axils varied from day to day and from morning to evening during dry periods and that the axils were often apparently dry for days at a time although the larvae were able to survive. It is suggested that the larvae are able to survive in the water film at the base of the axils, but that such unfavourable conditions may account for the retarded growth that has been observed.

The relationship between water content and number of larvae per axil is discussed, and the conclusion is reached that unless all measurements of water content for all varieties are made simultaneously each day, a comparison of average water content becomes impossible, and therefore no connection can be established from the data so far available.

Acknowledgement.

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EFFECT OF POST-TREATMENT TEMPERATURE ON INSECT RESISTANCE TO INSECTICIDAL SPRAYS.

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The effect of temperature on the resistance of insects to insecticidal treatment is of much theoretical interest to biologists in general and of much practical importance to applied entomologists. The toxicity of DDT in relation to temperature has been investigated by several workers using a variety of insects and methods of application. There are, however, only a few contributions in the case of the newer insecticides such as toxaphene, chlordane, parathion, etc. Further, the results of many workers are conflicting, demanding further observations and proper interpretation.

In an earlier publication (Pradhan, 1949b) an effort was made to formulate three basic generalisations, (a) that insect resistance to poisons changes with temperature, as in the case of other vital activities, increasing up to a critical point and afterwards declining, (b) that the amount of poison reaching the site of action also varies with temperature, generally but not always increasing with its rise, and (c) that the apparent effect of temperature on insecticidal action is the resultant of the effects of these two factors, namely, resistance and pick-up. As corollaries of these generalisations,* it was pointed out that (i) irrespective of the form in which the insecticide is administered, an increase of temperature within limits during treatment, *i.e.*, during exposure or contact, should be expected to increase the toxic effect, (ii) an increase of temperature within limits after treatment, *i.e.*, during the major part of the reaction, should decrease the toxic effect, and (iii) an increase of temperature before treatment should result both in an increase in resistance as well as in activity, the former decreasing the toxic effect and the latter likely to increase the pick-up.

With these generalisations as a working hypothesis, we have been differentiating, in our studies on the effect of temperature on insect mortality due to insecticides, the temperature factor into three components, *pre-treatment* temperature, *treatment* temperature, and *post-treatment* temperature. During the present investigations the effect of post-treatment temperature on resistance to insecticidal sprays is dealt with.

Material and Technique.

Test insect.

Tribolium castaneum (Hbst.), in the adult stage, was used as the test insect. Cultures of this insect were maintained in whole wheat flour on more or less the same lines as described by Tattersfield & Potter (1943). In each experiment, adults from the same culture or from cultures started on the same date were randomised and used.

Insecticides.

The insecticides used were (i) pure p,p'DDT (M.P. 108–109°C.) separated by partial crystallisation from a commercial product, (ii) γ BHC (lindane),

* Brown (1951, pp. 237–243), while reviewing the literature on the subject, has expressed more or less similar views.

(iii) toxaphene of technical grade with 67-69 per cent. chlorine content, (iv) technical grade of chlordane, and (v) parathion (pure).

Technique.

The experimental technique mainly consisted of pre-treating the insects at a comparatively low temperature (about 14°C.), then spraying the insects with different strengths of an insecticidal formulation and removing them to clean containers at different temperatures for reaction and observation at different intervals. This procedure therefore involved four major operations in addition to the assessment of toxic effect.

(i) *Pre-treatment of insects.*—All the insects to be used in a particular experiment (except that with emulsified solutions of DDT, see below) were pre-conditioned without food for 24 hours at a temperature of 14°C. \pm 1.5 to 2.0°C. in a glass desiccator maintained at 50 to 55 per cent. relative humidity by means of potassium hydroxide solution. This rather low temperature of about 14°C. was chosen for pre-conditioning because in our experience insects pre-conditioned at a low temperature give more consistent results, especially when differentiating the effects of temperature during treatment and post-treatment. A change in the pre-conditioning temperature may change the results.

(ii) *Insecticidal formulations.*—Six insecticidal formulations were tested, emulsified solutions of DDT, γ BHC, toxaphene, chlordane and parathion, and a suspension of DDT. Each formulation was applied in a range of five or six suitable concentrations. In the preparation of emulsified solutions, benzene was used as the solvent and Lissapol 370 as the emulsifier, their proportions being kept constant at 5 per cent. (v/v) and 0.625 per cent. (w/v), respectively, in each concentration of each insecticidal formulation. The emulsified solutions were prepared on the day they were to be used. DDT suspensions were formulated to give hexagonal crystals of 3.8 \times 13-15 microns in size; acetone was used as the solvent and Lissapol 370 as the wetter, their proportions being kept constant at 10 per cent. (v/v) and 0.2 per cent. (w/v), respectively, in the various concentrations.

(iii) *Application of insecticide.*—The sprays were applied by means of a laboratory apparatus based on the principles of the Potter tower (Potter, 1941). Clean petri dishes, 12.5 cm. in diameter, were used as containers for the test insects during spraying, with filter paper circles (Whatman No. 41, 12.5 cm. in diameter) as substrate in the dishes. Two cc. of spray liquid was used in each operation, the pressure being kept at 24 cm. of mercury. Except in the case of DDT emulsified solution (see Table I), the experiments with each formulation were carried out at all the temperatures with material from the same culture of insects conditioned at the one temperature and with the same preparation of insecticide. Fifteen replications, each of 10-12 insects, were sprayed with each of the five or six concentrations of each insecticidal formulation. Immediately after spraying, the insects were removed to clean untreated glass tubes (3" \times 1") open at both ends but with pieces of muslin stretched over the ends and held in position by means of rubber rings. For each insecticide or formulation of insecticide there were, therefore, either 75 or 90 tubes. These were divided into five lots (of 15 or 18 tubes each), each of which contained 3 tubes treated at each concentration. One such lot for each of the six insecticides or formulations of insecticide was kept at each of the five post-treatment temperatures in glass desiccators at 50-55 per cent. R.H.

(iv) *Post-treatment temperatures.*—The temperature of about 14°C. was maintained in a refrigerator and those between 20 and 40°C. in electrically heated incubators. The exact temperatures maintained in each experiment are given separately.

(v) *Assessment of toxic effect.*—For mortality counts the insects were removed

from the tubes to clean petri dishes, using a separate dish for each concentration, and assigned to one of the following five categories, normal, slightly affected, badly affected, moribund and dead (see Tattersfield & Potter, 1943; Pradhan, 1949*a*). After examination, the dead insects were discarded and the rest were returned to their respective tubes at their respective temperatures for further observations up to six days at about 48-hour intervals. In the analysis of data, the last two categories were taken together for calculating percentage mortality. An adjustment for mortalities in the control was made using Abbott's formula.

Results of Experiments.

Since the technique adopted was essentially the same in all the experiments, only the results obtained with each insecticidal formulation are recorded here (Table I). With the exception of the DDT emulsified-solution treatment, where

TABLE I.

LC50 (based on 4th-day observations).

	DDT emulsified solution	DDT suspension	γ BHC emulsified solution	Toxaphene emulsified solution	Chlordane emulsified solution	Parathion emulsified solution
No. of insects used	1440	1047	1050	1012	1225	1245
Post- treatment temperature (°C.)						
12 — 13	*0.032					
13 \pm 1	**0.035					
14 \pm 1.5		†0.0433	†0.470			
14 \pm 2				††1.205	††1.862	††0.01005
21 \pm 1	*0.109					
24 \pm 1		†0.1392				
24 \pm 2			†0.706	††1.167	††1.578	††0.00982
26 \pm 1	*0.149					
30	**0.204	†0.3083				††0.00741
30 \pm 0.5				††1.046		
31 \pm 1			†0.825		††1.199	
35	**0.133	†0.2004		††0.948	††0.729	††0.00873
35 \pm 0.5			†0.590			
40	**0.093	†0.0682	†0.582	††0.776	††0.537	††0.00615

Pre-conditioning temperatures (°C.) indicated as follows:—*, 12–13; **, 13 \pm 1; †, 14 \pm 1.5; ††, 14 \pm 2.

one half of the test insects was pre-conditioned at 12–13°C. and the other half at 18° ± 1°C., all tests with each insecticide were carried out with insects pre-conditioned at a single temperature.

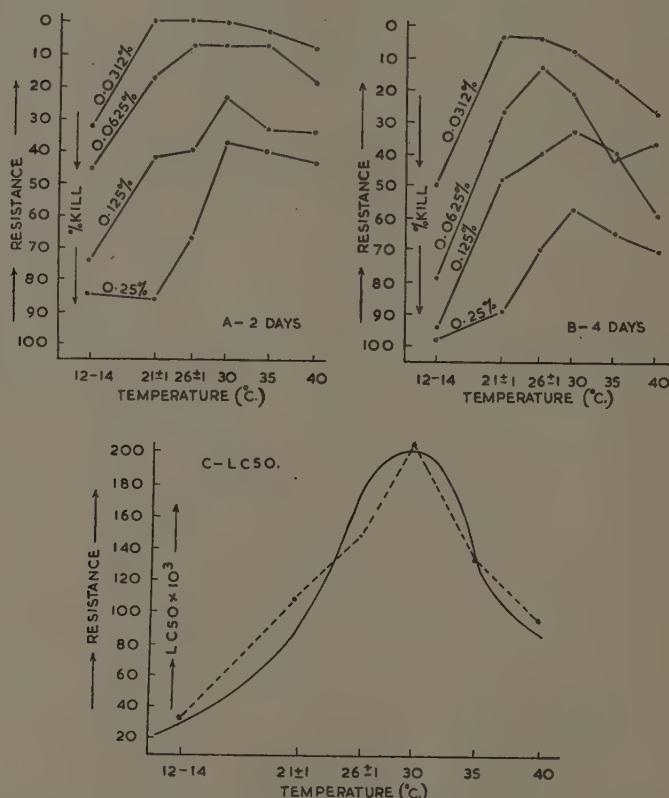


Fig. 1.—Effect of temperature on the mortality of adults of *T. castaneum* sprayed with emulsified solution of DDT. A and B illustrate observations on 2nd and 4th days, respectively, after the application of insecticides. Each graph is for one concentration. C illustrates LC50 values based on 4th-day observations. The broken line represents the actual values and the solid one a smooth theoretical curve.

Although in each experiment the mortality counts were continued up to six days, the results obtained on the fourth day were used to calculate the regression equations by probit method and to determine the values of LC50. For graphical representation it was found useful to make a deviation from the usual log-concentration-probit mortality graphs to what we may call temperature-mortality-percentage graphs for different concentrations and separately for each period of observation (figs. 1 A & B; 2 A & B; 3 A, B & C; 4 A, B & C; 5 A, B & C; 6 A, B & C). Also in these graphs, contrary to the usual practice, the mortality

percentage along the ordinate has been labelled from the top downwards. As a result of these slight modifications it will be seen that the graphs directly show the trend of temperature coefficient of resistance to insecticidal actions. The

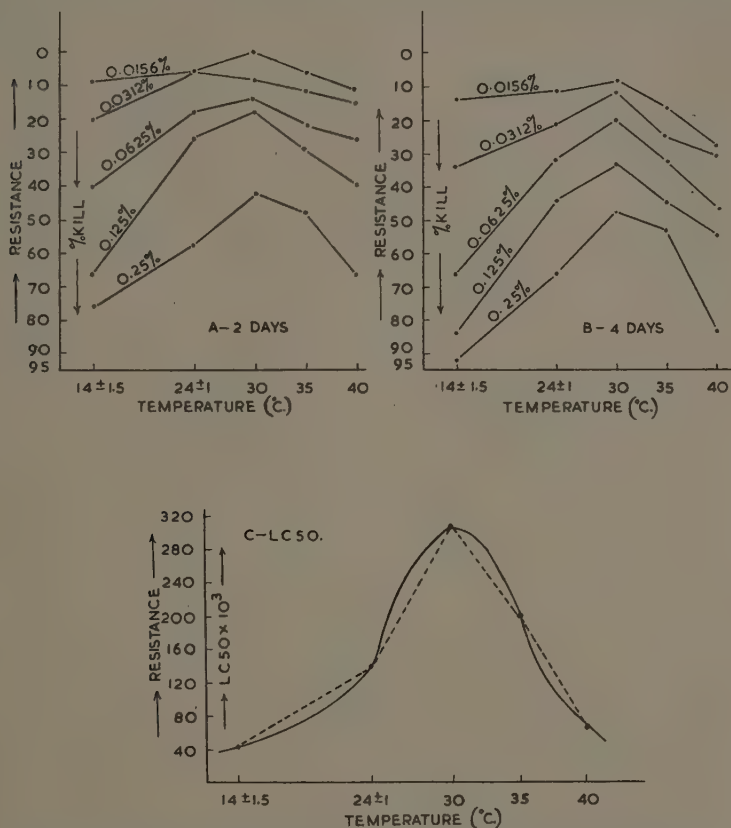


Fig. 2.—Effect of temperature on the mortality of adults of *T. castaneum* sprayed with DDT suspension. A and B illustrate observations on 2nd and 4th day and C the LC50 values based on 4th-day observations. Other details as in fig. 1.

same trend is also shown when the values of LC50 for each of the formulations are plotted against the temperatures under which they were obtained (figs. 1 C, 2 C, 3 D, 4 D, 5 D, & 6 D).

Discussion.

A study of mortality data given in figs. 1 to 6 will show that so far as DDT emulsified solutions (fig. 1 A & B), DDT suspensions (fig. 2 A & B) and γ BHC emulsified solutions (fig. 3 A, B & C) are concerned, the percentage mortality was lowest at a post-treatment temperature of about 30°C. and at temperatures above and below this point it became progressively higher. In the case of emulsified

solutions of toxaphene (fig. 4 A, B & C), chlordane (fig. 5 A, B & C) and parathion (fig. 6 A, B & C), the percentage mortality continued to increase with increase in post-treatment temperature throughout the range of temperature studied. As expected from these mortality trends, the graphs obtained by plotting the values of LC50 against the temperature under which they were obtained show the same trends.

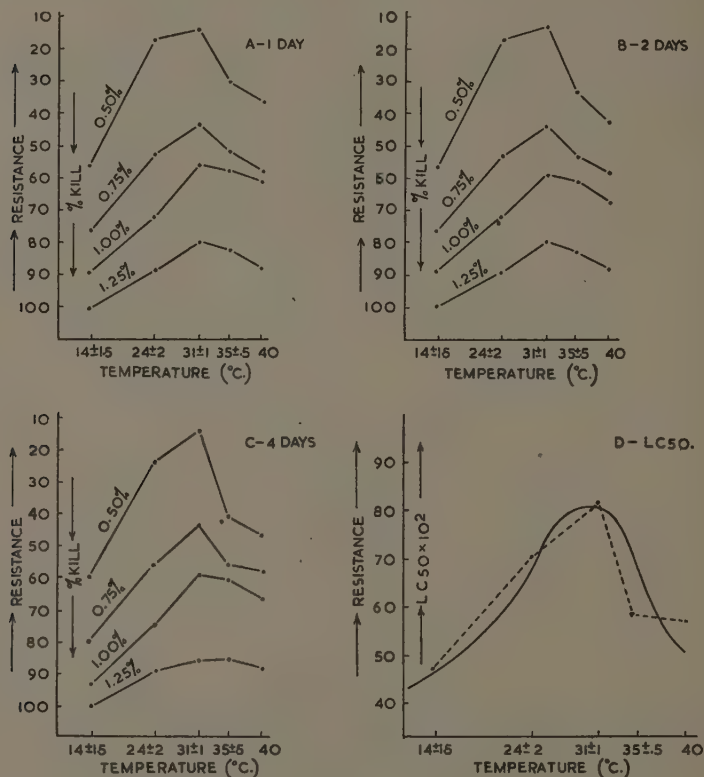


Fig. 3.—Effect of temperature on the mortality of adults of *T. castaneum* sprayed with emulsified solution of BHC. A, B and C illustrate observations on 1st, 2nd and 4th days and D the values of LC50 based on 4th-day observations. Other details as in fig. 1.

There are two important points arising out of these observations to which it is necessary to draw special attention.

It has already been suggested (p. 261) that an increase in the post-treatment temperature within limits (i.e., up to a particular temperature) should decrease the toxic effect. This expectation has been realised during the present investigation only in three (figs. 1, 2 & 3) out of six formulations or in only two (DDT and γ BHC) out of five insecticides. Possible explanations for such divergent results with different insecticides or with the same insecticide under different conditions require to be considered.

There is an interesting similarity between the curves obtained by plotting the values of LC50 for DDT and γ BHC formulations (figs. 1 C, 2 C & 3 D) against different post-treatment temperatures and the curves published in some cases by various workers relating temperature with various physiological activities. There are, however, relatively few such observations recorded in the literature and their significance is not always well understood.

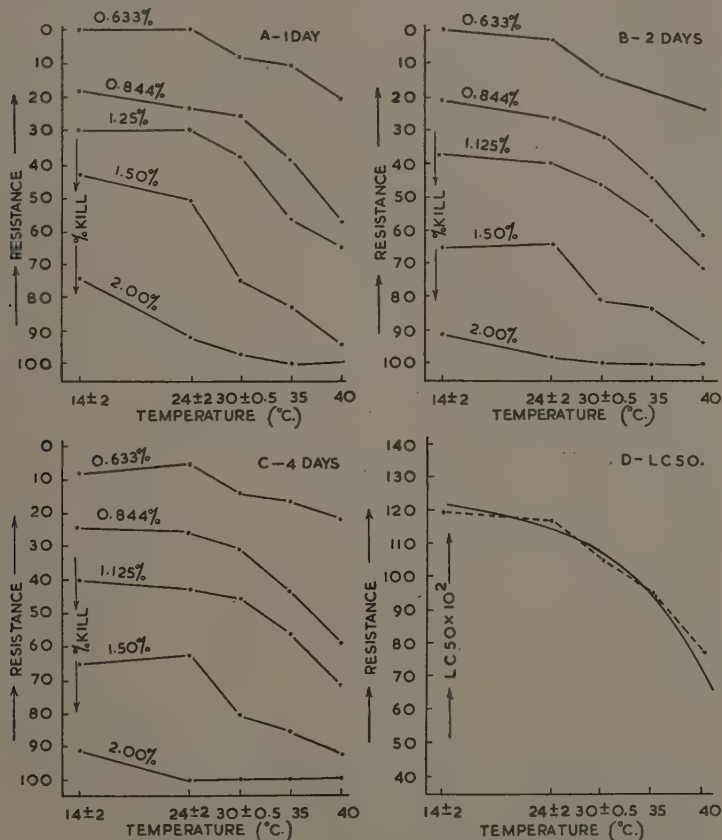


Fig. 4.—Effect of temperature on the mortality of adults of *T. castaneum* sprayed with emulsified solution of toxaphene. A, B and C illustrate observations on 1st, 2nd and 4th days and D the values of LC50 based on 4th-day observations. Other details as in fig. 1.

Positive or negative temperature coefficients with different insects or different insecticides.

A study of the literature, in addition to observations similar to those recorded in the present paper, even within the range where temperature exerts no lethal effect, shows that there are cases in which either a negative or a positive post-treatment temperature coefficient of insect mortality occurs. (A positive temperature coefficient of mortality is shown when an increase in mortality occurs with a rise in temperature, a negative one when a decrease occurs.) In

point of fact, both types of observation exist with different insects or with different insecticides, or sometimes with the same insect and the same insecticide but in different temperature ranges. Without attempting a complete review of all the work on the subject it may suffice to mention some examples of both these types of observations.

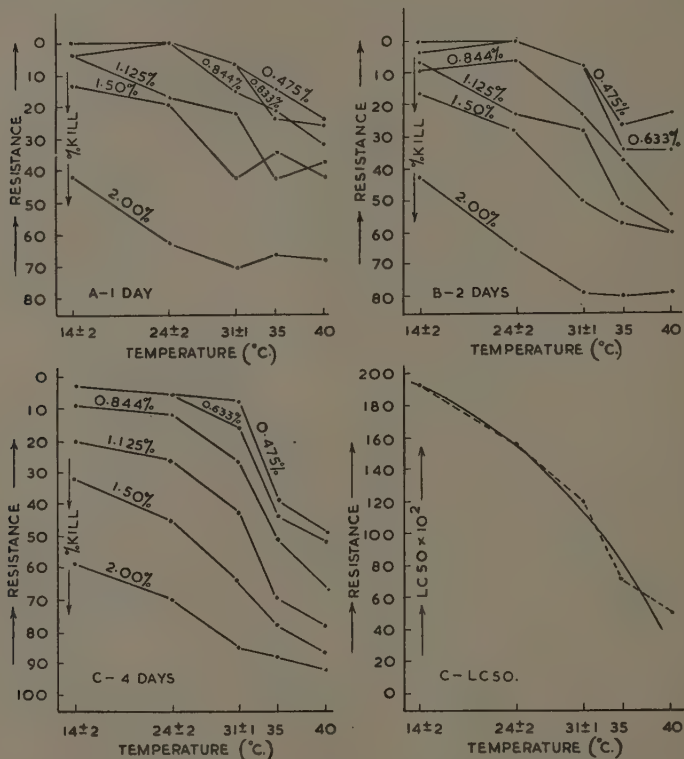


Fig. 5.—Effect of temperature on the mortality of adults of *T. castaneum* sprayed with emulsified solution of chlordane. A, B and C illustrate observations on 1st, 2nd and 4th days and D the values of LC50 based on 4th-day observations. Other details as in fig. 1.

DDT.—In the case of DDT, the insecticide has been applied in a variety of ways, i.e., as dusts, sprays, films, by immersion, and by injection, and a variety of test insects has been used: Lindquist & others (1945), house-flies; Rhoades & Brett (1948), three species of grasshoppers; Dustan (1947), larvae of *Plutella maculipennis* (Curt.) and *Phlyctaenia rubigalis* (Gn.); Pradhan (1949b), adults of *Tribolium castaneum* and larvae of *Plutella maculipennis*; LaPlante (1949), larvae of *Cydia molesta* (Busck); Häfliger (1948, 1949), honey bees (stomach poison); Hoffman & Lindquist (1949), house-flies (keeping the same temperature both before and after treatment); Guthrie (1950), *Blattella germanica* (L.); McIntosh (1951), adults of *T. castaneum* and *Oryzaephilus surinamensis* (L.); Vinson & Kearns (1952), *Periplaneta americana* (L.); and Roth, Lindquist &

Terriere (1953), house-flies. All these workers recorded a negative temperature coefficient although some have used temperatures up to 100°F. (38°C.); there were, however, indications of this negative temperature coefficient tending to become positive in two instances (Dustan, 1947; Pradhan, 1949b). A negative temperature coefficient has also been reported by Hoffman, Roth & Lindquist

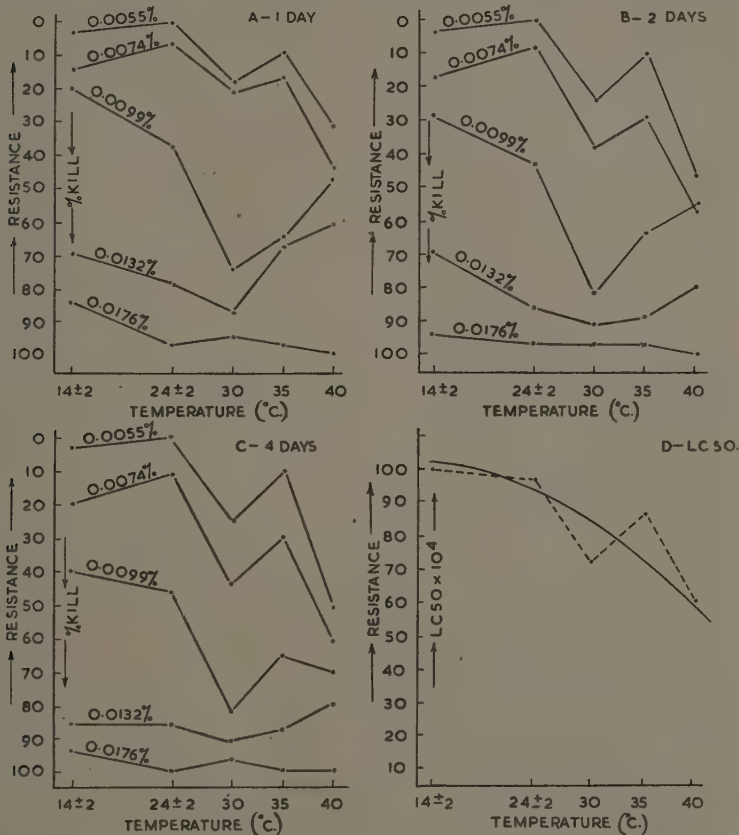


Fig. 6.—Effect of temperature on the mortality of adults of *T. castaneum* sprayed with emulsified solution of parathion. A, B and C illustrate observations on 1st, 2nd and 4th days and D the LC50 values based on 4th-day observations. Other details as in fig. 1.

(1949) and Guthrie (1950) although they maintained the same temperature before and after the application of the insecticide. On the other hand, Woodruff (1950) using injected doses of DDT against nymphs and adults of *Oncopeltus fasciatus* (Dall.) observed a positive temperature coefficient over a lower range of temperatures (10 to 22°C.) and a negative temperature coefficient over a higher range of temperature (22 to 29°C.). Another type of observation has been reported by Fan, Chen & Richards (1948) who studied the effect of temperature at different concentrations of DDT and found that (a) following external application, the temperature coefficient was either positive or negative according to the DDT

concentration or dosage; at high concentrations it was positive, at low concentrations negative and (b) following injection, a positive temperature coefficient was always obtained irrespective of dosage.

Other insecticides.—Rhoades & Brett (1948) studied the effect of post-treatment temperature on the action of γ BHC, toxaphene, chlordane and parathion on three species of the grasshopper, *Melanoplus*. For all the four insecticides these workers observed a positive temperature coefficient of insecticidal action between 70° and 100°F. (21°–38°C.). Häfliger (1949) studied the effect of post-treatment temperature on the action of parathion, γ BHC and calcium arsenate fed to honey bees. He found a maximum resistance at 28°C. for the first two insecticides although the effect of temperature on the kill was very small; in the case of calcium arsenate the coefficient was slightly positive throughout the range of temperature tested. In our laboratory the γ BHC dust used against adults of *T. castaneum* has given a negative temperature coefficient of action at post-treatment temperatures between 12° and 27°C. (Pradhan & Srivastava, unpublished). Guthrie (1950), by maintaining the same temperature before and after the treatment, reported a negative temperature coefficient when γ BHC or pyrethrum was applied topically to adult female cockroaches, although the results were rather variable in the case of BHC; but he found a positive coefficient in the case of aldrin and dieldrin. Hoffman, Roth & Lindquist (1949) and Hoffman & Lindquist (1949) maintained the same temperature before and after treatment of sheep keds, *Melophagus ovinus* (L.), and house-flies, respectively. With methoxychlor and DDD they observed a negative temperature coefficient in both the sheep ked and the house-fly; with γ BHC, however, there was a positive coefficient in the case of sheep ked, but in the case of house-fly the coefficient appeared to depend on the dose of the insecticide (also *vide* Pradhan, 1950), it being positive at 200 mg./sq. ft., zero at 50 mg./sq. ft. and negative at 3 mg./sq. ft. Chlordane and toxaphene gave a positive coefficient with both sheep ked and house-fly. Four other insecticides (heptachlor, parathion, dieldrin and aldrin) were tried on the house-fly and all gave positive coefficients. Böttcher (1938, 1939) obtained negative temperature coefficients with pyrethrum and derris and a positive temperature coefficient in the case of nicotine, all the three being used as stomach poisons against the honey bee. Potter & Gillham (1946) observed a negative temperature coefficient in the case of pyrethrins and nicotine used as spray against adults of *T. castaneum*. Woodruff (1950) studied the temperature coefficients of the action of insecticides when injected. The coefficient was positive with nicotine in the case of nymphs as mortality was greater and survival less at higher temperature, but in the case of adults the coefficient appeared to be negative, taking the survival figures into consideration (as there was greater survival at higher temperatures), and positive, taking the mortality figures into consideration. The coefficient with sodium azide was positive for nymphs and zero for adults, but with rotenone it was positive both for nymphs and adults. A negative coefficient of action has been reported in the case of pyrethrum, used in topical application or as spray against a variety of insects by Klinger (1936), against *Eutettix tenellus* (Baker) by Harries, DeCoursey & Hofmaster (1945), and against house-fly by Eagleson (1942), who also obtained similar results with lethane.

Fumigants.—The literature on the temperature coefficient of the action of fumigants has been reviewed recently by Pradhan & Govindan (1954) and the position is more or less the same as that described above.

Possible explanation.

A positive temperature coefficient of insect mortality has been generally explained firstly on the basis of increased metabolism or increased chemical action at higher temperatures and secondly on the basis of quicker penetration of the

insecticide into the insect body or its quicker arrival at the site of action in some other way at higher temperatures. On the other hand, a negative temperature coefficient of insect mortality due to insecticides has been explained mainly on the basis of increased physiological resistance to the insecticide at higher temperatures. But physical and chemical factors have been suggested as well, such as greater detoxification of the insecticide inside the insect body at higher temperatures, greater adsorption of the insecticide at a lower temperature, or even the toxic effect of very low temperature itself.

Taking all these views into consideration, one might offer the following possible explanation for the occurrence of both positive and negative temperature coefficients. The factors inducing both positive and negative temperature coefficients are operative in each case. Sometimes the former predominate, whilst on other occasions it is the latter, depending on the test insect used and the nature and concentration (Pradhan, 1950) of the insecticide as well as on the technique of experimentation. Generally speaking, factors such as pick-up, penetration, etc., leading to a positive temperature coefficient of mortality are predominant and it requires special experimental techniques, even in the case of a most suitable insecticide like DDT, to eliminate the masking effect of them and to bring out the effects of physiological resistance which lead to a negative temperature coefficient. This fact was demonstrated by Pradhan (1949b) when a positive temperature coefficient of the action of DDT films (in the case of continuous contact of the insect with the insecticide film) was first observed, but later, when the contact period was reduced, the pick-up factor was eliminated and a negative temperature coefficient, which could be explained on the basis of increased physiological resistance, was evident. If this line of thought is correct, then it is not impossible that in the case of toxaphene, chlordane, and parathion, the techniques used so far have failed to eliminate the effects of penetration, etc., and what we are reporting in the case of these insecticides may still be a result of the two opposing sets of factors. Finer techniques may have to be evolved to detect in such cases the negative temperature coefficient due to physiological resistance of the insect. These might be achieved by adjusting the duration and extent of the contact of the insect with the insecticide in so delicate a manner that the insect will pick up a dose such that poisoning of its system will not proceed beyond the possibility of recovery. Until a more critical technique is evolved, the above possibility must be regarded as purely tentative and conjectural. If all reasonable attempts fail to detect the negative temperature coefficient of insect mortality, due to physiological resistance in the test insects in the case of chlordane, parathion, etc., then the hypothesis proposed will have to be abandoned. However, in the absence of any more convincing explanation, supported by experimental observations, the foregoing tentative suggestion has been put forward because it offers a possible explanation of the diverse types of observations. On somewhat similar lines it was possible to explain the observations of Fan, Chen & Richards (1948). These authors explained their observations by concluding that, at low concentrations, DDT penetrates the arthropod cuticle more effectively at low temperatures. Pradhan (1950), however, interpreted the same observations differently, but without presuming any fundamental reversal in the action or penetration of DDT when its concentration changes from high to low. According to him it appeared that the temperature coefficient of DDT entry remained positive irrespective of concentration, but that its value decreased with decrease in concentration and became masked by the temperature coefficient of insect resistance when a negative temperature coefficient of DDT action began to be observed in actual experiments. A partially parallel case is offered by the work of Shepard, Lindgren & Thomas (1937), on the effect of temperature on the median lethal concentration of ethylene dichloride to *Tribolium confusum* Duv. In this case the MLC continued to increase from 0 to 10°C., then decreased from

10 to 20°C. and then again began to show a slight rise from 20 to 35°C. Thus here also there is a positive temperature coefficient of insect mortality from 10° to 20°C., as in the case of Woodruff's observations, followed by a very slightly negative temperature coefficient. Pradhan (1949b) explained this phenomenon in the following way. He thought that the increase in median lethal concentration from 0° to 10°C. indicated the increase in the insect's resistance with the rise in temperature. This upward trend of resistance would have probably continued beyond 10°C., but an increase in temperature means also an increase in insect activity (especially respiration in the case of fumigant), resulting in increased dosage of the fumigant being taken in by the insect. This increased dosage not only masked the upward trend of resistance but actually reversed it, apparently indicating increased susceptibility with rise in temperature. Thus it is possible that in the case of Woodruff's insects also, the effect of increase in physiological resistance due to increase in temperature from 10° to 22°C. was not strong enough to avoid being masked by those factors which are responsible for the arrival of the insecticide at the site of action, and which tend to bring about a positive temperature coefficient of insect mortality due to insecticidal action.

Similarity between temperature coefficient of insect resistance to insecticides and temperature coefficient of other physiological activities.

One of the interesting points brought out by the present investigations is an essential similarity between the curves obtained by plotting the values of LC50 for DDT and γ BHC formulations (figs. 1 C, 2 C & 3 D) against the different temperatures, and the curves, published by various workers, relating temperature with other physiological activities such as development, movement, etc. Assuming that under the conditions of the experiment the values of LC50 can be taken as a true index, it is clear that the resistance to DDT and γ BHC formulations increases with the increase in temperature up to a certain point and then decreases with further rise of temperature, the highest points in the aforesaid figures representing the temperature at which the insect can offer the best resistance to the toxic action of the insecticide. It is interesting to note that these points are not far from the general optimum temperatures for various other physiological activities. Such clear-cut curves as are illustrated in figs. 1 C, 2 C and 3 D are quite rare in the literature, although Häffiger's (1949) data do indicate higher resistance of the honey bee to the stomach-poison effect of parathion and γ BHC at 28°C. than at either 20° or 36°C. and also there is an indication of the negative temperature coefficient tending to become positive at higher temperatures in the data published by Pradhan (1949b) and Dustan (1947). Dustan, however, ascribed this change possibly to rapid drying of the leaves at higher temperatures.

Overall position.—Although the results with DDT and BHC could be explained by the theory put forward, it is difficult to apply it to give a satisfactory explanation of the results obtained with the other insecticides. It is hoped that the present paper may stimulate efforts to plan more critical experiments and lead to a substantiation of the theory or the reverse.

Summary.

Results are given of investigations on the effects of five post-treatment temperatures on the mortality of adults of *Tribolium castaneum* (Hbst.) sprayed with five or six concentrations of each of six insecticidal formulations, emulsified solutions of DDT, γ BHC, toxaphene, chlordane and parathion and a suspension of DDT.

With both emulsified solutions and suspensions of DDT and emulsified solutions of γ BHC the mortality of the insects decreased with the rise of temperature

from about 14° to 30°C. and increased when the temperature increased from 30° and 40°C. On the other hand, with emulsified solutions of toxaphene, chlordane and parathion, the mortality of the test insects increased continuously with increase of temperature from 14° to 40°C. As a possible explanation for such divergent results with different insecticides it is tentatively suggested that whilst the inherent physiological resistance of *Tribolium* to DDT and γ BHC formulations which appear to increase with increase of temperature up to a certain point has been demonstrated, the technique has not been sufficiently critical in the case of the other formulations, and it is thought that physiological resistance has possibly remained masked by other factors and that the values of LC50 are not a true index of physiological resistance in the case of these insecticides.

An essential similarity between the curve obtained by plotting values of LC50 against temperature in the case of DDT and γ BHC and those relating temperature to other physiological activities (published by various workers) has been stressed, implying a similarity between the resistance of the insect to insecticides on the one hand and its other physiological activities on the other.

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OBSERVATIONS ON THE BEHAVIOUR OF CULICINE MOSQUITOS IN AFRICAN HUTS.

By JOHN PHIPPS, M.Sc., D.I.C.*

For several years prior to 1953 the Tanga Branch of the Ross Institute of Tropical Hygiene was carrying out anti-malarial measures on sisal estates in the Tanga Province of Tanganyika Territory. The method used mainly was hut-spraying at three-monthly intervals with water-dispersible formulations of γ BHC. Considerable success was attained in the control of species of *Anopheles*, but Culicine mosquitos proved refractory and continued to be a serious nuisance. Numbers were normally reduced for 2-3 weeks after treatment, but after this as many mosquitos as before entered huts and fed on the occupants. With the object of reducing this nuisance, trials with dieldrin were instituted on estates near Tanga and, as a preliminary to the application of the insecticide, observations were made from November 1952 to July 1953 in mud huts with palm-thatch roofs on Pongwe Sisal Estate (Amboni Estates Ltd.) near Tanga.

Methods.

The huts used, which had never before been treated with any insecticide, were situated between two treated camps and approximately one mile from each. They were built by Africans who had retired after long service on the estate and were occupied by the same people continuously. There were about 30 huts in the group, of which 24 were chosen as suitable for observations. A square opening was cut in the east wall of each sleeping room (or that nearest to east), to take a window trap of the usual type, consisting of mosquito netting over a one-foot wooden cube frame with a funnel opening to trap mosquitos leaving the hut. There were no other openings in the hut walls except the door, and only narrow gaps below the eaves. Doors are usually kept shut in African huts and are always shut at night, so that it may be assumed that most of the mosquitos leaving the huts were taken in the traps.

Catches were made in four huts each day, so that each hut was used only once a week. A window trap was fixed in place at 2 p.m. and was removed at 6 p.m.; the catch of mosquitos was termed the "evening trap". The window trap was then replaced by another which was removed at 8 a.m. the following day, this catch being termed the "morning trap". The square opening was then closed by a board, sheets were laid on the floor, a pyrethrum-in-kerosene aerosol was applied to the interior space of the hut and the mosquitos that fell on to the sheet collected. These are referred to as the "spray catch". As each hut was treated once a week the repellent effect of the kerosene had time to wear off between observations (Muirhead-Thomson, 1951). The sequence of catches for each hut was thus evening trap, morning trap, spray catch. All times quoted are East African Time. As Tanga lies in 5°S. latitude, times of sunrise and sunset do not differ by more than 15 minutes each way from 6 a.m. and 6 p.m., respectively, throughout the year.

The work on the estate was done by a trained African and was checked by the author three or four times each month. The mosquitos taken were sent to the Ross Institute laboratory in Tanga.

All the Culicine mosquitos examined were found to be *Culex pipiens fatigans*

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Wied., and while not more than one-fifth were closely examined by the author, it seems unlikely that any other species was present in any considerable numbers. The African engaged in sorting had instructions to set aside for examination any which appeared to belong to other species, but did not in fact find any. Some examples of *Anopheles* were taken, mostly of *A. gambiae* Giles, but not enough to make analysis of distribution worth while.

The *Culex pipiens* complex has been discussed by Mattingly & others (1951). The form discussed in the present paper bites man to such an extent as to be a serious nuisance, and it appears to prefer to feed indoors. The numbers taken by spray catch in some areas may be as high as 1,000–2,000 females per room and in these circumstances the occupants of the huts usually sleep outside for comfort. The larvae are found everywhere, but are present in enormous numbers in unsealed septic tanks and pit latrines and in sisal waste water. This water is always present in abundance, as it is used for washing the fibre during decortication. It contains a high concentration of decomposing plant material. The domestic habit of *fatigans* has been stressed by Deane (1951) in Brazil and by Wharton (1951) in Malaya. Haddow (1942) did not find it an important member of the hut fauna at Kisumu, Kenya, but in the lower-lying parts of East Africa, and especially on sisal estates, it is the most important nuisance mosquito. Harris (1942) mentions that *fatigans* breeds in all manner of places, including cess-pits, and that adults are especially obvious in huts during the dry season.

The total numbers of Culicines taken in these observations were 12,810 females and 5,446 males. The catch was also sorted into stages of the gonotrophic cycle as described below. This work was done by another African under the supervision of the author. It was not difficult to recognise the stage even in mosquitos which had been dead for some days. The mosquitos were staged by external examination only, as time did not permit of dissection. It cannot, therefore, be claimed that the stages correspond with those proposed by Covell (in Christophers, Sinton & Covell, 1939) for *Anopheles*, which depend basically on the deposition of yolk in the eggs and on the development of the floats. It is perhaps unfortunate to use the term "stage", in view of the definitions given by Covell, but the use of "blood-fed", "half-gravid" and "gravid" is also open to objections in this connection, as will be pointed out below. The original concepts of Covell were modified for staging *Anopheles* by external examination by Hocking & MacInnes (1948), whose definitions were made use of in the work reported here, staging being based on the figures on p. 455 of their paper. During the course of observations carried out in the Tanga area in 1949, dissections had been made of *C. p. fatigans* and the external appearance correlated with the progress of the gonotrophic cycle. The following correspondences between external appearance and internal condition can be definitely stated:—

Stage I. *External*—no blood, no visible ovaries, abdomen thin. *Internal*—stomach empty, ovaries small, follicles without yolk.

Stage II. *External*—abdomen distended by blood, ovaries occupying not more than 3 segments dorsally and 2 ventrally. *Internal*—some yolk granules in follicles.

Stage III. *External*—blood still obvious, ovaries occupying not more than 6 segments dorsally and 4 ventrally. *Internal*—yolk granules occupying more than half the follicle.

Stage IV. *External*—little blood visible, ovaries occupying all but 0–1 segments dorsally and occupying 3–4 ventrally. *Internal*—eggs full of yolk.

Stage V. *External*—stomach not visible or visible only as a narrow black line on the ventral surface, abdomen distended by ovaries. *Internal*—eggs fully developed.

The following qualifications to the above scheme must be mentioned. Mosquitos are very occasionally taken with stage I ovaries and with the abdomen

distended by water, or plant juices, contained in the stomach. These were classed as stage I, but did not amount to more than 1 per cent. of the stage I taken. In the dissections mentioned above, it was sometimes noted that stage I ovaries contained a few fully formed eggs. These represent, of course, mosquitos which have returned to stage I after oviposition, but as this has in any case been assumed the correlation is not invalidated.

It is uncertain how many blood-meals are taken during the cycle. A second meal, by distending the stomach and compressing or obscuring the ovaries, may cause a mosquito to be classified in a stage earlier than its true ovarian stage, except, of course, for stage I. Thus, the final effect would be that stage II would appear to be more numerous and later stages less numerous than would be the case if the mosquitos were dissected and classified on ovarian development alone. However, what was here being studied, where stages were concerned, was the effect on behaviour of the taking and digestion of a blood-meal and of the development of the ovaries. This process is essentially a continuous one except for the transitions from stage I to stage II (taking of blood-meal) and from stage V to stage I (oviposition), and it is precisely these stages which can be recognised with certainty by external examination. A mosquito with, say, stage III ovaries which has just taken a second blood-meal may behave like a stage II or a stage III mosquito, but provided the numbers studied are large enough the results may be expected to be correct for the population as a whole.

The difficulty is not surmounted by classification as "blood-fed", "half-gravid" and "gravid" (Muirhead-Thomson, 1951), since a half-gravid mosquito that takes a second meal could be regarded either as blood-fed or as half-gravid. It should also be remembered that "gravid" has a precise meaning used for animals other than mosquitos, and its application to mosquitos with stage II ovaries in order to fit the time of digestion of the blood-meal, as proposed by Gillies (1954), can only lead to confusion.

To summarise the interpretation of stages adopted in the present paper: stage I includes mosquitos that have not fed and that have undeveloped ovaries; stage II, those that have fed recently and in which ovarian development has not proceeded very far; and stage V, those with ripe eggs and little or no blood; while stages III and IV are intermediate between II and V as regards digestion and ovarian development.

Results and Analysis.

A summary of the results week by week, without division according to huts, is presented in Table I. The various columns give the sub-totals, for each week, of the females (in each stage) and males taken by spray catch and in the two traps, and the weekly totals for each sex; the last line of the Table gives the totals of the above over the whole period of the observations. The results, subdivided in the same way, are summarised for each hut, without division into weeks, in Table III.

The full data on which these summaries are based, which are too extensive for reproduction here,* were subjected to an analysis of variance. In the analysis the minimum period of time used was one week, as a week's observations include catches in all huts and it is not possible to subdivide the week without confounding differences between days with those between huts. There are thus in the analysis 24 huts, each used once a week for 31 weeks, and the mosquitos were taken in each hut either in the evening trap, morning trap or by spray catch. These last three divisions are referred to in the analysis and subsequent discussion as "places". The female mosquitos taken in each place were classified into stages

* Copies of additional summaries of the data, attached to a reprint of this paper, have been deposited in the library of the Commonwealth Institute of Entomology.

TABLE I.
Numbers of Culicine mosquitoes, with stages for females, caught in huts and in exit traps in successive weeks, 1952-53.

Week no., ending	Spray catch						Morning trap						Evening trap						Weekly totals		Rain- fall (mm.)					
	Females						Males	Females						Males	Females							Males				
	I	II	III	IV	V	Total		I	II	III	IV	V	Total		I	II	III	IV	V	Total						
1 9.xii	107	245	44	49	48	491	29	316	0	2	4	7	16	341	96	252	0	2	12	5	281	43	1098	78	0.0	
2 16.xii	189	238	50	49	46	407	46	352	2	0	4	5	41	411	28	238	0	0	4	31	91	261	113	134	0.0	
3 23.xii	46	166	59	52	34	377	40	382	0	0	23	0	2	119	28	82	0	0	11	24	128	66	622	131	0.0	
4 30.xii	42	187	34	17	14	294	57	96	0	0	5	2	2	103	23	117	0	0	7	30	154	33	551	116	0.0	
5 6.i	29	142	28	36	12	247	42	59	0	0	3	8	3	63	52	75	0	0	1	5	81	22	391	116	39.0	
6 14.i	22	102	23	19	9	175	58	59	3	6	7	14	8	89	35	41	0	0	2	19	62	47	326	140	0.0	
7 21.i	46	66	11	15	2	140	50	53	0	0	0	2	7	62	47	55	0	0	0	6	43	43	265	170	0.0	
8 28.i	27	159	23	25	10	244	52	56	0	0	0	4	7	67	83	69	0	0	0	11	49	77	403	255	0.9	
9 4.ii	39	174	20	20	9	253	158	47	0	0	0	0	10	67	83	69	0	0	0	0	18	35	262	209	0.6	
10 11.ii	40	102	9	7	3	161	102	47	0	0	0	0	2	49	72	44	0	0	0	0	5	41	58	231	111	0.0
11 18.ii	54	92	5	4	8	158	74	56	1	2	0	0	4	63	56	57	0	0	0	1	10	51	113	337	407	24.7
12 25.ii	32	148	24	6	5	215	148	62	0	1	2	6	7	146	40	0	0	0	1	10	51	113	337	407	24.7	
13 5.iii	10	106	13	2	38	169	64	9	4	4	0	0	27	44	72	12	0	2	0	31	46	81	258	217	0.8	
14 12.iii	16	60	9	10	8	103	24	22	0	0	1	1	24	31	29	0	0	0	0	4	33	36	160	91	33.2	
15 18.iii	33	77	1	13	5	119	46	47	0	0	0	3	10	60	63	43	0	0	0	13	92	42	241	151	49.0	
16 25.iii	26	129	29	18	6	208	84	56	0	0	0	0	0	56	64	46	0	0	0	0	40	311	138	2.4		
17 3.iv	38	305	20	15	1	379	35	88	0	0	0	0	2	90	111	74	0	0	0	8	77	75	546	221	49.4	
18 10.iv	39	319	29	18	3	385	50	80	0	0	0	0	0	89	107	74	0	0	0	2	65	81	419	289	58.4	
19 15.iv	34	179	24	13	8	252	54	85	0	0	0	0	2	87	107	70	0	0	0	4	74	81	413	242	22.3	
20 22.iv	79	223	27	24	14	327	155	178	0	0	0	0	20	198	246	103	0	0	0	11	115	141	680	542	24.3	
21 29.iv	31	232	32	35	15	325	45	139	0	0	0	1	14	154	196	104	7	0	7	16	134	124	613	365	65.7	
22 6.v	13	249	45	26	13	346	22	83	4	0	0	1	14	102	58	63	2	0	0	19	84	42	532	122	180.9	
23 13.v	33	221	21	14	5	299	31	200	0	0	0	0	0	209	102	145	0	0	0	19	164	61	672	194	28.6	
24 20.v	36	126	11	2	29	204	89	37	0	0	0	1	11	49	77	45	0	0	0	15	61	64	314	210	130.0	
25 27.v	18	175	12	9	19	233	29	41	0	0	0	0	8	49	77	45	0	0	1	0	10	56	71	338	177	15.7
26 3.vi	9	145	30	12	20	142	3	55	1	1	2	8	5	51	34	37	0	0	0	4	21	68	23	247	80	28.5
27 10.vi	35	142	86	31	12	256	21	82	0	2	5	17	106	37	50	0	0	0	3	4	21	77	39	439	97	6.0
28 17.vi	5	30	9	12	2	67	8	34	3	0	0	1	5	48	45	34	0	0	0	4	41	48	151	96	0.0	
29 24.vi	6	63	6	1	7	83	19	27	0	0	0	0	0	37	24	14	0	0	0	7	21	30	141	73	0.0	
30 1.vii	3	59	13	9	9	71	105	11	1	0	0	0	17	17	14	0	0	0	6	20	21	142	46	0.0		
31 8.vii	10	70	19	13	13	10	122	6	30	1	0	1	11	43	39	27	2	0	0	17	46	48	211	93	0.0	
Totals	1046	4523	686	565	386	7206	1512	2563	19	22	81	306	2891	2207	2144	24	5	61	379	2613	1727	12310	5446			

I-V of the gonotrophic cycle, but this subdivision does not increase the number of degrees of freedom available in the analysis for the calculation of the effects of huts, places and weeks on the female mosquito population as a whole. Two estimates of the error mean square must therefore be made for females, one for sums over stages based on the dispersion of the original numbers (error *a*) and one for the classes after subdivision into stages (error *b*). The first of these is the huts \times weeks \times places interaction and the second the huts \times weeks \times places \times stages interaction. This argument is taken from Paterson (1939). The analysis of males does not, of course, involve subdivision into stages and was made independently.

There are thus 24 huts \times 31 weeks \times 3 places \times 5 stages = 11,160 observations in all in the analysis of the females, and 2,232 in that of the males, giving 11,159 and 2,231 degrees of freedom, respectively. The subdivisions of these, together with the results of the analyses, are set out in Table II.

TABLE II.

Analysis of variance of captures of male and female mosquitos.

Females					
	Sum of squares	Degrees of freedom	Mean square	Variance ratio	P
Huts	259.19	23	11.27	2.6565	< .001
Weeks	5245.58	30	174.85	41.219	< .001
Places	3408.14	2	1704.07	401.67	< .001
Huts \times Weeks	3355.09	690	4.86	1.141	> .05
Huts \times Places	211.54	46	4.60	1.084	> .05
Weeks \times Places	663.71	60	11.06	2.607	< .001
Error (<i>a</i>)	5854.41	1380	4.242	—	—
Stages	10610.67	4	2652.67	694.4	< .001
Stages \times Huts	256.74	92	2.79	—	—
Stages \times Weeks	7214.21	120	60.35	15.79	< .001
Stages \times Places	17027.92	8	2128.49	557.19	< .001
Stages \times Huts \times Weeks	11194.15	2760	4.056	1.06	> .01
Stages \times Huts \times Places	851.47	184	4.627	1.21	< .01
Stages \times Weeks \times Places	6070.53	240	25.29	6.62	< .001
Error (<i>b</i>)	21085.91	5520	3.82	—	—
Males					
Huts	258.07	23	11.22	1.7209	> .01
Weeks	4783.66	30	159.455	24.456	< .001
Places	426.91	2	213.455	32.739	< .001
Huts \times Weeks	6490.56	690	9.406	1.4426	> .05
Weeks \times Places	1284.14	60	21.402	3.2824	< .001
Huts \times Places	280.53	46	6.099	—	—
Error	8998.09	1380	6.52	—	—

The results will be considered in the order which seems most relevant to the development of the argument. In this type of work, in which detailed personal supervision of the collection and classification was not possible, there are even more sources of error than is usual in biological observations generally. In case these errors were not random, a value of $P = 0.01$ has been regarded as probably significant and $P < 0.001$ as highly significant.

Differences in attractiveness between huts.

The total number of females caught per hut varied from 441 to 619, and that of males from 171 to 287. The variation in the attractiveness of different huts

was significant in the case of females (least significant difference 120 at $P = 0.01$), but not in that of males. The result for females is not surprising. Differences in attractiveness of the huts may depend on meteorological conditions inside them or on the attractiveness to female mosquitos of the people inhabiting them. Dakshinamurty & Sharma (1951a) showed that *fatigans* prefers the higher of two humidities offered, except where this is saturated air. They further showed (1951b) that it prefers a temperature of 25°C. to one of 30°C. Muirhead-Thomson (1938) also found a strong avoidance of high temperatures, most pronounced in hungry females, but found this species indifferent to humidity differences except near saturation, where all except hungry females are sensitive to a difference of 1 per cent. R.H. These observations suggest that meteorological conditions inside a hut may affect mosquito behaviour, and it is possible that the response is also influenced by outdoor conditions. However, male mosquitos do not enter huts to feed and the fact that differences for males are not significant suggests that, unless males are indifferent to some climatic stimuli that affect females, the attractiveness of the inhabitants may be the more important factor influencing female behaviour. The ranking of huts according to numbers of females is not correlated with the ranking according to males, and this would suggest that the two sexes are responding differently.

It is noteworthy that males were much less numerous than females throughout the observations. Qutubuddin (1953) showed that the sex ratio for *fatigans* at emergence is 1:1 and, unless the female is much the longer lived, it can only be supposed that a higher proportion of males than of females rests outdoors and never enters huts.

Numbers of mosquitos remaining in and leaving huts.

Differences between numbers taken in traps and by spray catch are highly significant for both sexes, significant differences between the totals shown in Table I being 281 for females and 348 for males, for a value of $P = 0.01$. There is, however, a great difference in the behaviour of the two sexes. Females are most numerous in spray catches (7,206), least numerous in the evening trap (2,613), while males are most numerous in the morning trap (2,207) and least numerous in the spray catches (1,512), though the difference between these and the evening trap catches (1,727) is not significant.

The use of an evening trap was suggested by Davidson (1953), and it is interesting to note that considerable numbers of both sexes were taken in it in these studies. Had it not been used, all these mosquitos would have been missed and rather different results for females would have been obtained, as appears from the discussion of stages, below. It must also be noted that while the morning trap was in place for 14 hours, from 6 p.m. to 8 a.m., which includes the hours when mosquito activity is most obvious, the evening trap was in place for only 4 hours, from 2 to 6 p.m. Apart from the trap catches, there is little evidence as to periods of mosquito activity, but some evidence is brought forward below to support the view that *fatigans* is inactive during the hours of daylight. If this is so, then the mosquitos in the evening trap must have entered it during quite a short period before 6 p.m., and the rate of hut leaving at this time must be considerably greater than during the night as a whole.

At first sight the results suggest that males may behave similarly to stage I females. A contingency test, however, shows that the distributions differ significantly. Since males frequently leave huts during the night or early morning, the suggestion made above that outdoor resting is more usual in males than in females receives some support. There is no significant difference between catches of males in huts and in evening traps, which suggests that those males that do rest in huts leave in the early evening. Probable movements of stage I females at this time are discussed later. It is difficult to suggest a reason for hut entry

TABLE III.

Totals of Culicine mosquitos taken in different huts, 1952-53.

Hut no.	Spray catch										Morning trap										Evening trap					Total females	Total males
	Females					Males					Females					Males											
	I	II	III	IV	V	Total	I	II	III	IV	V	Total	I	II	III	IV	V	Total									
1	47	211	40	31	92	351	74	124	79	153	12	68	2	0	1	8	79	64	583	217							
2	42	131	25	13	253	68	143	101	58	127	16	58	0	0	0	14	74	59	489	228							
3	43	191	30	15	17	296	63	107	82	162	13	69	0	0	3	11	83	51	506	196							
4	34	244	22	26	16	342	54	88	65	120	20	109	0	0	0	14	79	62	480	171							
5	25	183	32	27	20	231	42	131	121	20	7	152	0	0	2	18	142	80	598	238							
6	46	243	27	20	345	64	122	101	112	15	141	106	0	0	3	13	133	78	608	294							
7	46	245	20	16	20	347	60	82	99	4	15	99	0	0	4	14	109	86	586	233							
8	45	192	38	26	17	327	60	91	110	4	13	110	78	0	0	21	131	86	520	248							
9	54	145	28	24	15	238	68	133	161	0	4	141	96	0	1	102	102	61	552	239							
10	59	142	38	34	17	290	82	142	115	0	4	150	94	0	0	16	140	70	539	242							
11	41	186	29	26	21	303	78	115	110	4	16	150	94	0	1	15	143	80	552	268							
12	34	183	23	28	11	275	63	115	115	0	4	16	150	94	0	16	143	70	539	242							
13	32	161	25	11	21	213	36	98	121	5	3	130	140	0	2	3	138	66	506	269							
14	30	206	32	14	513	36	110	92	92	0	2	83	104	0	0	10	79	61	521	195							
15	50	224	39	28	14	307	53	99	139	0	5	16	120	0	0	13	129	65	519	178							
16	50	224	38	25	16	353	33	99	139	0	2	83	104	0	0	13	129	65	519	178							
17	48	173	21	23	21	286	68	106	111	0	4	16	131	0	0	13	96	68	568	183							
18	83	164	12	18	16	293	104	112	131	1	0	18	134	0	0	5	123	61	553	237							
19	37	164	42	23	17	283	73	109	118	0	0	12	118	0	0	3	102	72	495	268							
20	56	172	31	18	13	290	79	101	120	1	1	12	118	0	0	23	118	80	592	248							
21	48	208	80	26	11	323	78	87	131	0	0	11	114	0	0	22	123	75	547	234							
22	38	207	25	27	14	258	49	116	160	0	3	18	131	0	0	19	138	88	486	200							
23	42	189	28	25	14	295	49	112	160	0	0	18	131	0	0	19	138	88	486	200							
24	44	165	15	14	7	245	44	82	117	0	0	4	137	0	0	15	104	78	441	204							
Totals	1046	4523	686	565	386	7206	1512	2563	19	22	81	306	2901	2207	2144	24	5	61	379	2613	1727	12810	5440				

by males that do not rest there during the day, the time when it might be expected that comparatively cool, dark places would be sought, but the possibility cannot be excluded that mating occurs in huts. In Mattingly & others (1951), it is pointed out by Mattingly that one strain of *fatigans* will breed readily in cages, by Rozeboom that *C. quinquefasciatus* Say (the name preferred by most American authors for *fatigans*) will breed in small cages, and by Knight, quoting Tate & Vincent (1936), that in *C. p. molestus* Forsk., another member of the *C. pipiens* complex, males copulate with resting females.

The results for females, of which many more were taken by spray catches than in either trap, can be explained only on the assumption that most females spend more than one day in a hut. Deane (1951), using a morning trap and spray catching for *fatigans* in Brazil, took 2,222 females in huts, out of a total of 2,270 caught. Wharton (1951), using the same technique, found that most *fatigans* remained in the huts, the proportion in the window trap varying from 10-60 per cent., with an average for all observations of 28 per cent. In the present series the morning trap catch, expressed as a percentage of the morning trap + spray catch, gives 29.3 per cent., and Wharton's proportion thus agrees with the present series of observations.

Variations in mosquito catches with time.

Total catches for each week are set out in Table I. Significant differences are 87 for females and 108 for males, for a value of $P = 0.01$. The ranking of the different weeks according to the numbers of females taken is significantly correlated with that according to males. The fall in total numbers of mosquitoes after the second week was not caused by the killing of large numbers at the outset of catching, for the observations in this series were in fact begun on 5th November, although results obtained before 3rd December are not considered, because no evening trap was used until then.

Deane (1951) notes that, in Brazil, *fatigans* occurs all the year round with a peak at the height of the wet season. Harris (1942) found it especially obvious in huts during the dry season. Certainly, on sisal estates in Tanganyika, *fatigans* is most troublesome during the dry season, and this, it is suggested, is due to its preference for breeding in sisal waste water. As mentioned above, this water is always present in abundance, and the absence of rain makes no check to breeding. During the rains, considerable flushing occurs, which is often assisted on estates by the clearance of vegetation to enable the accumulated waste to be carried away, and this must wash out enormous numbers of larvae.

Rainfall records are included in Table I, from which it appears that there is no obvious relation between mosquito numbers and rainfall. In 1953, the long rains lasted from March to May, finishing somewhat earlier than usual, but the amounts were about what is usual for the season.

Frequencies of mosquitos in successive stages of the gonotrophic cycle.

The total numbers of female Culicines in the various stages of the gonotrophic cycle (with the approximate percentages in brackets) were as follows: stage I, 5,753 (45); stage II, 4,566 (35.6); stage III, 713 (5.5); stage IV, 707 (5.5) and stage V, 1,071 (8.4). A difference of 463 was significant at $P = 0.01$.

These numbers might be used to estimate the fraction of the adult life spent in each stage amongst that part of the population that haunts huts, if it could be assumed that deaths occur at random. The numbers taken differ considerably from those given by Hocking & MacInnes (1948) for *Anopheles*, and from all published figures which it has been possible to consult, in the high proportion of stage I. It would appear that almost half the adult life is spent in this stage, indicating that there is considerable delay between emergence or oviposition and feeding. Stage II, as judged in these observations, must last almost as long, and

this would suggest that several blood-meals are taken during the development of the ovaries, *i.e.*, that a considerable proportion of mosquitos assigned to stage II had ovaries that were, in fact, in later stages of development. If this were so, then the digestion of the final blood-meal would be accompanied by the last stages of the maturation of the ovaries, which would, at the same time, rapidly become more apparent externally.

In view of these results, it is particularly unfortunate that no window traps were used between 8 a.m. and 2 p.m., without which it is impossible to be quite certain that fed females did not leave huts in this period. However, it seems extremely unlikely that they did so, in view of the very small numbers of stage II taken in traps throughout the observations, discussed in the next section. During five years in Tanganyika, I have carried out spray catches in many hundreds of huts similar to those used in the observations, usually during the morning. Some of them yielded thousands of *fatigans*, many of them fed, and, after spraying, the humming of mosquitos was very loud indeed. Before spraying nothing could be heard and there was no indication of the large numbers present. It seems most unlikely that in the present observations enough mosquitos were missed at any time to account for the discrepancies in numbers between the stages.

The effect on behaviour of the stage in the gonotrophic cycle.

This is measured by the stages \times places interaction, which is highly significant. Totals of different stages caught in huts and in exit traps are given in Table I (bottom line), a difference of 267 being significant at $P = 0.01$. It is apparent that catches of stages II, III and IV are significantly greatest in huts; stage I is most numerous in the morning trap, while there is no significant difference between the numbers of stage V taken in huts and in the two traps.

The simplest interpretation of these results is that both gravid females and hungry ones are restless, while fed females are sluggish. The sequence of events would thus be as follows. A newly emerged female, being hungry and therefore restless, moves about until it encounters a stimulus attractive to it in its physiological state. This may be the scent of a host or air of another temperature or humidity emanating from a hut, or both. After entry it may or may not be stimulated to feed—that many mosquitos leave huts without feeding is shown by the high proportion of stage I in the exit traps. This also supports the conclusion drawn from the relative numbers of the stages as to their relative durations. If the female feeds, its restlessness passes and it settles down during digestion of the meal, so long as the microclimate offered is not too repellent. After a time the mosquito becomes hungry again and takes another meal, usually in the same hut, otherwise it would have been expected that larger numbers of stages III and IV would have been taken in traps. As the ovaries develop with digestion of the final meal, restlessness returns gradually (the proportions leaving huts increase in stages IV and V) and the physiological state is such that it is attracted again outside the hut. In this reaction light may be important. After oviposition the cycle is repeated.

Some observations bearing on the effect of stage on hut-entry and hut-leaving have been made on other mosquitos, mainly species of *Anopheles*. They tend to show that *A. gambiae* and *A. funestus* Giles enter huts with an empty stomach, that most of them feed and that those leaving are in late ovarian stages. Haddow (1954), using the ovarian Stages of Christophers, Sinton & Covell (1936), has stated that Culicines biting in the open do so late in Stage I or early in Stage II, which is broadly what has been assumed here. He also notes that some Culicines, normally living outdoors and taking only one blood-meal in each cycle, will take repeated meals in captivity, and it may be that in this respect the behaviour of *fatigans* in huts resembles that of other Culicines in captivity.

Variations in the numbers of the different stages with time.

This is measured by the interaction stages \times weeks, which is highly significant. Figures may be obtained from Table I, by adding the three entries for each stage in each week. The difference for significance is 83 for $P = 0.01$. Weekly total numbers in the different stages vary as follows: stage I, from 31 in week 13 to 677 in week 2; stage II, from 42 in week 28 to 305 in week 17; stage III, from 1 in week 15 to 59 in week 3; stage IV, from 1 in week 29 to 86 in week 3; stage V, from 6 in week 17 to 100 in week 2. Thus all stages except III are significantly more numerous at some times than at others, and not all of their maxima or minima fall in the same week. This result cannot be related to the gonotrophic cycle, for the unit of time is one week and each week's catches represent the sum of six daily catches. Therefore, unless the cycle occupies considerably more than one week, which is unlikely, all weekly sums should include approximately the same proportion of each stage.

It has frequently been noted that the elimination of a mass breeding site, e.g., the closure of an open septic tank, is followed by a dramatic disappearance of mosquitos from the neighbourhood, suggesting that the rate of loss of mosquitos from huts is high. If this daily loss, due to some unknown factor, varied during the 31 weeks of the observations, it might be possible to explain the variation in the proportions of the stages with time. It may be noted that, in stages III, IV and V, numbers were greatest in either week 2 or 3, which might have been a time when losses were low.

It is further notable that although in the nine weeks in which the total catch was more than 500 mosquitos, both stages I and II were always significantly more numerous than III, IV or V, in six of the seven weeks with a total catch less than 250, neither I nor II was significantly more numerous than any of the other stages. Further, in these six weeks, both stages I and II were fewer than the lowest numbers taken in the nine weeks with high catches. Stage III, however, was never significantly more numerous in the nine weeks than in the seven, and IV and V show only one value each, in two different weeks, where the values in the nine weeks exceed any of those in the seven. Thus, low total catches are associated with a deficiency of stages I and II, but not of the other stages, which suggests that low catches are due to a lower rate of reproduction—and perhaps fewer blood-meals in the gonotrophic cycle, to account for the decrease in stage II. Five of these seven weeks occurred in June and July, at and after the end of the rains, when temperatures are lower and when *fatigans* is normally least troublesome. Either way, the observed effect must be attributed to the action of an external factor, whether reduced breeding or increased loss.

Variations in behaviour in different weeks.

Variations in behaviour in different weeks can be estimated, for either sex, from the totals columns for the captures in huts and in exit traps, given in Table I. The effect is measured by the places \times weeks interaction in Table II, which is highly significant for both sexes, least significant differences being 50 for females and 62 for males, for $P = 0.01$.

As regards females, the result must be considered in relation to the effect of stage on behaviour and the variations in the proportions of the different stages with time. In fact, the weeks in which exit traps accounted for the highest proportion amongst the female captures were those with the highest proportions of stage I or late stages or the lowest proportions of stage II.

Amongst the males, there was no significant difference between spray catch, morning trap and evening trap in 24 of the 31 weeks. There were significantly more males in the morning trap than in the huts in weeks 17, 19, 20, 21, 23 and 24, while in week 10 the spray catch was significantly greater than the evening trap catch but there was no difference between the morning trap catch and spray

catch. In the six weeks in which the tendency to leave huts was most marked there was some rain, though there were other weeks with rain in which there was no marked tendency for males to leave huts. There was no rain in week 10. It seems possible, therefore, that there is some sort of relation between male behaviour and outside humidity.

Variations in the attractiveness of a hut with time, in the distribution of stages amongst huts and in the tendency for mosquitos of all stages to leave different huts.

These are measured by the interactions huts \times weeks, stages \times huts and huts \times places. None of them is significant for either sex (the second, of course, not being applicable to males). In other words, the huts of the group did not change in their relative attractiveness during the observations, the proportions of the stages were not significantly different in different huts and the proportions remaining in and leaving huts were the same for all huts.

Relations among three factors taken together.

Only three factors altogether are considered for males, but in the case of females, second-order interactions, of three factors taken together, can be

TABLE IV.

Approximate percentages of each stage (I-V) of female Culicine mosquitos taken in three places in successive weeks, 1952-53.

Week no.	Spray catch					Morning trap					Evening trap				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
1	16	100	95	82	69	47	0	5	12	23	37	0	0	6	8
2	13	99	77	61	28	51	0	15	22	41	35	1	8	17	31
3	22	94	100	61	57	39	1	0	27	19	39	5	0	12	24
4	16	100	100	59	30	38	0	0	17	4	46	0	0	24	66
5	7	100	100	90	50	33	0	0	8	33	60	0	0	2	17
6	18	97	79	68	21	47	3	21	25	33	35	0	0	7	46
7	30	100	100	79	13	34	0	0	10	47	36	0	0	11	40
8	21	100	100	100	50	44	0	0	0	24	35	0	0	0	26
9	24	100	100	95	0	34	0	0	5	50	42	0	0	0	50
10	30	100	100	100	23	35	0	0	0	15	35	0	0	0	62
11	32	99	71	100	37	33	1	29	0	48	35	0	0	0	15
12	23	100	96	66	24	46	0	4	22	28	31	0	0	11	48
13	33	97	68	100	40	29	3	21	0	28	38	0	11	0	32
14	24	100	100	90	61	33	0	0	9	9	43	0	0	0	30
15	25	100	100	50	18	36	0	0	50	35	31	0	0	0	47
16	20	100	100	100	85	44	0	0	0	0	36	0	0	0	15
17	19	100	100	100	17	44	0	0	0	34	37	0	0	0	49
18	19	100	100	100	32	47	0	0	0	44	34	0	0	0	24
19	17	100	100	100	32	45	0	0	0	24	38	0	0	0	44
20	22	100	100	96	31	49	0	0	0	44	29	0	0	1	25
21	11	96	100	81	33	51	0	0	3	31	38	4	0	16	36
22	8	96	100	98	28	52	3	0	2	30	40	1	0	0	42
23	10	100	100	100	15	52	0	0	0	27	38	0	0	0	58
24	30	99	100	66	53	31	0	0	33	20	39	1	0	0	27
25	17	100	94	100	50	40	0	0	0	22	43	0	6	0	23
26	12	99	97	77	24	32	1	3	8	21	56	0	0	15	55
27	21	100	95	74	24	49	0	5	12	34	30	0	0	14	42
28	6	80	100	75	18	47	20	0	7	46	47	0	0	18	36
29	13	100	100	100	29	57	0	0	0	42	30	0	0	0	29
30	11	100	100	100	62	37	0	0	0	20	52	0	0	0	18
31	15	96	100	93	26	45	1	0	7	28	40	3	0	0	46

examined, of which the only one that is highly significant is stages \times weeks \times places. Figures are given in Table I, the difference for significance being 48 at $P = 0.01$. The significance of the interaction indicates that the distribution of stages between different places of capture changes with time. Percentages of each stage in huts and traps are set out in Table IV. Highest and lowest percentages taken in huts were: stage I, 33-6; stage II, 100-94; stage III, 100-68; stage IV, 100-50; stage V, 69-0. Thus, stages II and III are most closely tied to huts, and stage I is the least; stage IV leaves fairly often, and the most variable behaviour is shown by stage V.

Discussion.

Much of the work now being done on the evaluation of residual insecticides depends on the study of spray and trap catches. It is highly desirable that more should be known about behaviour in the absence of any insecticide, so that the effect of the insecticide can be more precisely evaluated. The use of specially built huts may present mosquitos with conditions quite different from those of huts typical of the area concerned, and may modify behaviour. If the mosquitos are not staged, an unusual preponderance of one stage at the time of observation could give a false impression as regards house-frequenting habits. Similarly, the effect of outdoor climate at different times of the year will also be different.

Where assessment of the results of an insecticide trial depends on counting dead mosquitos no such criticism can be made, but where it depends on relative numbers caught inside the hut and in exit traps, serious errors may creep in. The use of a control hut will not always eliminate these, for two huts which appear similar may be entirely different to the mosquito.

Summary.

Observations on the behaviour of *Culex pipiens fatigans* Wied. were made in 24 huts built and occupied by Africans on a sisal estate near Tanga, Tanganyika Territory. Catches were made in four huts each day, an exit trap being fixed on the window from 2 p.m. to 6 p.m. (evening trap) and replaced by another from 6 p.m. to 8 a.m. next day (morning trap), when a spray catch was made of the mosquitos still in the hut. Each hut was used only once a week, for 31 weeks, from December 1952 to July 1953. The females caught were sorted according to the stage of the gonotrophic cycle, as judged by external examination. The stages used were: I, no blood, no visible ovaries; II, stomach full of blood, ovaries small; V, little or no blood, ovaries fully developed; III and IV, intermediate between II and V.

An analysis of variance was performed on the results. Catches of both sexes showed a significant variation with time, which was not, however, correlated with rainfall. Significantly different numbers were taken in the traps and in the huts. Females were significantly most numerous in the hut catch, and males in the morning trap, the former result suggesting that most females spend more than one day in a hut. There were significant differences between the numbers of females in the different stages of the gonotrophic cycle, stages I and II together accounting for 80 per cent. of all females caught. The high proportion of stage I is attributed to delay between emergence and feeding, and of stage II to the taking of more than one meal during the gonotrophic cycle, later meals obscuring the state of ovarian development. There were significant differences between huts as regards the numbers of females caught, but not as regards males, and it is concluded that this is due to variations in the attractiveness of the inhabitants of the huts.

Females in stage I were most numerous in the morning trap, those in stages II-IV were taken only rarely in traps, but there was no significant difference

between numbers of stage V caught in huts and in the two window traps. These results are attributed to restlessness in both hungry and gravid females, and sluggishness in fed ones. All stages except III were significantly more numerous in some weeks than in others and this might be due to variations in either rate of reproduction, or rate of loss of mosquitos from huts. There were significant variations in behaviour in different weeks. For females, this is attributed to changes in the proportions of the stages with time and the dependence of behaviour on stage. For males, there is some evidence of a relation between behaviour and humidity outside the hut. For females, the distribution of stages between different places of capture changes with time, stages II and III being most consistently associated with hut catches, while the most variable behaviour is shown by stage V.

The necessity for behaviour studies on mosquitos in the field is pointed out, especially in relation to field trials of residual insecticides.

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STUDIES ON THE CHOICE OF FOOD-PLANT AND CERTAIN ASPECTS
OF THE DIGESTIVE PHYSIOLOGY OF THE LARVAE AND ADULTS
OF *ATHALIA LUGENS PROXIMA* (KLUG) AND *EPILACHNA*
VIGINTIOCTOPUNCTATA (F.).*

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Relations of insects to their food supply are very diverse and complex. Food and feeding habits bear a direct relation to the morphology and physiology of insects. In the present paper the factors responsible for choice of food-plant have been briefly considered and some aspects of the physiology of digestion in *Athalia lugens* subsp. *proxima* (Klug) and *Epilachna vigintioctopunctata* (F.) have been studied.

Feeding Habits.

Athalia lugens proxima, the Mustard Sawfly, is mainly a nursery pest of cruciferous plants, the chief food-plants being *Raphanus sativus* (radish), *Brassica rapa* (turnip), *B. nigra* (mustard), *B. juncea* (rai), *B. oleracea* var. *gongylodes* (kohlrabi), *B. oleracea* var. *botritis* (cauliflower) and *B. oleracea* var. *bullata* (cabbage). Immediately after hatching, the larvae start feeding on the tender leaves, making holes of different sizes near the margin and centre. In extreme cases, they eat away practically the whole of the lamina, leaving only the veins and veinlets.

It is an observed fact that cauliflower, cabbage and kohlrabi are attacked by larvae and adults in the nursery only and most other food-plants also in the young state only, the one exception being turnip, the leaves of which are attacked even when the plants are fairly well grown; but as soon as young crops of alternative food-plants are available the adults migrate to them, feed on them and deposit most of their eggs in the tissues of these plants. On one occasion it was observed in the field that young mustard plants were heavily infested with the larvae of *A. l. proxima* although alternative food-plants, including turnip, were available nearby but were much older. Experiments in the laboratory and the field were planned to investigate food-plant selection in the Mustard Sawfly. In the laboratory, a set of leaves of almost equal size, and of practically the same age, of the food-plants listed above was put in each of five large petri dishes, towards the periphery, and larvae of almost uniform age were liberated in the centre so that all the leaves were more or less equidistant from the larvae. When the leaves were examined next morning, after an interval of 24 hours, maximum feeding was found to have taken place on the turnip leaves. In the field, a test for selective feeding habit was made by sowing the seeds of the seven food-plants mixed in one plot (5' x 5') and in another similar and adjacent one in individual patches. After a month, the maximum and the minimum infestations were noted to be on turnip and cabbage, respectively. This suggests that in the case of the attack on mustard noted in the field and mentioned above, age of the plant, and not its taste, probably played the major rôle. In this insect the choice of leaves of a particular group of plants can reasonably be attributed to odour and the preference for turnip leaves, to the more important rôle of taste.

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The Coccinellid, *Epilachna vigintioctopunctata*, is a common pest of solanaceous plants, the chief food-plants being *Solanum melongena* (brinjal), *S. tuberosum* (potato), *S. nigrum* (nightshade) and *Lycopersicum esculentum* (tomato). The larvae and adults feed on the leaves, the larvae usually on the lower surface and the adults on the upper, making fairly regular patches in the leaves, leaving slender and parallel strips of uneaten leaf lamina between them. In extreme cases the leaf finally withers and dries up.

As in the Mustard Sawfly, so in this insect also, it appears that odour and taste both help in the selection of food, taste being important, for it shows a distinct preference for brinjal, both in the field and the laboratory. To study the selective feeding habits, a replicated laboratory experiment was carried out on the lines of that used for *A. l. proxima*, and after an interval of 24 hours, maximum feeding was found to have taken place on the brinjal leaves. Fields where brinjal, potato and tomato grew side by side, and where nightshade plants occurred as a field weed, were examined in a number of localities at Allahabad, and the insect was noted to exercise a distinct preference for the leaves of brinjal. The fact that *E. vigintioctopunctata* is restricted in its food-plants to a particular group of plants and its observed preference for brinjal show that in this insect both odour and taste are important. Unlike *A. l. proxima*, age of the food-plant has practically nothing to do with the selection of food in this insect because larvae and adults have been observed feeding upon the leaves of mature and young plants more or less to the same extent.

TABLE I.

pH of different regions of *A. l. proxima* and *E. vigintioctopunctata*.

Region	Insect			
	<i>Athalia</i>		<i>Epilachna</i>	
	Larva	Adult	Larva	Adult
Blood	6.6	6.6	6.4	6.4
Salivary gland ..	6.4-6.6	6.2-6.4	5.4	6.6-6.8
Foregut	6.4-6.8	6.4-6.6	6.4-6.8	6.2
Midgut	6.6-6.8	6.4-6.6	6.0	6.0
Hindgut	7.0	6.6	6.0	5.4-5.7
Excreta	6.8	6.8	6.8-7.0	6.8-7.0

pH of leaves of different food-plants of *A. l. proxima* and *E. vigintioctopunctata*.

Insect	Food-plant (leaf)	pH
<i>Athalia</i>	radish	7.0-7.2
	turnip	7.0-7.2
	mustard	7.0-7.2
	rai	7.0-7.2
	cauliflower	6.8-7.0
	cabbage	6.8-7.0
	kohlrabi	7.0
<i>Epilachna</i>	brinjal	6.6-6.8
	potato	6.4-6.6
	tomato	6.6
	nightshade	6.8-7.0

Determination of Hydrogen-ion Concentration in Salivary Glands and in different Parts of the Gut.

The determination of hydrogen-ion concentration in various parts of the digestive tract is an important aspect of the study of the process of digestion, for different enzymes act under different hydrogen-ion concentrations.

As the quantity of secretion available from the gut wall is extremely small, the pH comparator and the pH meter methods were abandoned in favour of an indicator-paper method. Merck's indicator paper was used and only conductivity water was used whenever necessary, in the determination of pH.

The indicator-paper feeding method did not give satisfactory results as the larvae did not feed on the paper indicator. The larvae and adults were dissected (after about four to six hours' starvation to clear the gut of its food contents) and the different parts of the alimentary canal and other organs were slightly teased and brought into contact with indicator papers of different ranges. The results obtained are given, together with those from the leaves of different food-plants of the two insects, in Table I. Although there is no great difference between the pH of the alimentary canal of either insect and of the leaves of its respective food-plants, in neither are they identical.

Qualitative Estimation of Enzymes.

In order to prepare extracts of the various parts of the alimentary canal, larvae and adults were chloroformed and dissected immediately under distilled water. Salivary glands and different parts of the alimentary canal were taken out and ground with glycerine. Microtubes were filled with this material to about one-third, the remaining two-thirds being filled with toluene.

In each of the tests, described below, there were at least five replicates.

Amylase.

*Test * 1.*—Two drops of the tissue suspension of salivary gland, foregut, midgut and hindgut from larvae and adults of the two species were taken in separate microtubes while corresponding boiled suspensions were placed in separate microtubes as controls. To each of these were added two drops of 0.5 per cent. boiled soluble starch solution and the rest of the tube filled with toluene and incubated for from 72 to 96 hours to enable any decomposition of starch to be completed. After 96 hours the potassium iodide-iodine test was performed. The contents of the control tubes and the unboiled extracts of foregut and hindgut of *Athalia* and *Epilachna* and of the salivary gland of adults of *Epilachna* turned blue. The unboiled microtubes of salivary glands and midgut of larvae and adults of *Athalia* and of the salivary glands and midgut of larvae of *Epilachna* and of the midgut of adults of *Epilachna* did not turn blue. It is concluded, therefore, that amylase is present in the salivary glands and midgut of the larvae and adults of *A. l. proxima* and in the salivary glands and midgut of the larvae and in the midgut of adults of *E. vigintioctopunctata*.

Test 2.—The solutions which did not show blue colour with potassium iodide-iodine solution (incubated solutions of salivary gland and midgut of larvae and adults of *Athalia* and of larvae of *Epilachna* and of midgut of adults of *Epilachna*) were further tested for the presence of maltose—the decomposition product—by the picramic acid test. Four drops of the incubated solution, 1 drop of 10 per cent. sodium hydroxide solution and 2 drops of a saturated aqueous solution of picric acid were placed in a microtube in the order mentioned. The tube was then placed in an electric oven at 60°C. The colour of the yellow picric acid was changed to reddish-brown picramic acid. This indicates that maltose is formed

* Tests for proteases were performed on the lines of Hinman (1933) and the rest on the lines of Swingle (1928).

as a result of hydrolysis of starch by the amylase present in the salivary glands and midgut of the larvae and adults of *Athalia* and larvae of *Epilachna* and in the midgut of adults of *Epilachna*.

For further confirmation, Fehling's test and Fluckiger's test for sugar were performed in other sets of the incubated samples.

A few drops of Fehling's solution no. I were taken in a test tube and added to Fehling's solution no. II drop by drop till the precipitate that appeared in the beginning disappeared. To a few drops of the mixed Fehling's solution two drops of the incubated solution which had given test for the presence of amylase were added separately and heated and allowed to stand. Within five minutes of standing after heating, reddish-brown precipitate of copper appeared, indicating the presence of sugar.

In the case of Fluckiger's test, a drop of 20 per cent. sodium hydroxide was mixed with an equal quantity of powdered copper tartrate upon a slide until the copper salt was dissolved. A drop of each sample of incubated starch solution was added and the slide heated gently. A red precipitate of copper appeared, showing that reducing sugars were present.

Thus, all subsequent tests confirmed the presence of amylase in the salivary glands and midgut of larvae and adults of *Athalia* and of larvae of *Epilachna* and in the midgut of adults of *Epilachna*.

Maltase.

Test 1.—A 15 per cent. maltose solution was incubated with the tissue suspensions of different parts of the gut of the larvae and adults of *A. l. proxima* and *E. vigintioctopunctata*. After 48 hours or more of incubation, a drop was tested for the presence of glucose by the osazone test. One gm. of phenyl hydrazine hydrochloride was ground with 10 cc. of glycerine till dissolved and was then filtered through glass-wool. One gm. of sodium acetate was dissolved in 10 cc. glycerine. A drop of phenyl hydrazine hydrochloride solution was mixed with a drop of sodium acetate upon a slide, a drop of the solution to be tested was added, and a cover glass was put on. The slide was heated upon a water-bath at 100°C. for 15 minutes and allowed to cool. An hour after cooling, glucose-osazone appeared in the incubated tissue suspensions (from both larvae and adults) of midgut alone. Maltose had therefore been hydrolysed by maltase to glucose, indicating that maltase is present in suspensions of the midgut of larvae and adults.

Test 2.—Barfoed's test for monosaccharides was performed. A small quantity of acetic acid was added to a solution of copper acetate which is used to restrain the action of disaccharide. A reddish-brown precipitate appeared, on heating, in the midgut extracts of *Athalia* and *Epilachna*, showing that maltase is present.

Invertase.

A 15 per cent. sucrose solution was incubated with the tissue suspensions of gut. After incubating for 48 hours or more, a drop was tested for the presence of fructose and glucose by the osazone test and for reducing sugars by Fluckiger's test. Fructose-osazone appeared immediately and glucose-osazone upon cooling in midgut extracts whilst fore- and hindgut extracts gave negative results. Invertase, therefore, is present only in the midgut of *Athalia* and *Epilachna*.

Lactase.

A few drops of 15 per cent. lactose solution were incubated with the tissue suspensions of gut for 90 hours. The material was tested, after incubation, for glucose, by the osazone test and glucose-osazone appeared, an hour after cooling, in midgut extracts, showing that lactase was present.

Lipase.

The B.T.B. emulsion test was used to indicate the presence or absence of lipase. Four parts of olive oil and 2 parts of gum acacia were ground to a paste in a mortar; 3 parts of water were added, and the whole was triturated till a smooth emulsion was formed. A few drops of bromothymol blue were added to the emulsion and just sufficient of 2 per cent. sodium hydroxide to give it blue colour (pH 7.2). Similarly, the tissue suspensions of gut were adjusted to the same pH and added to the emulsion in a microtube. The blue colour gradually changed to green or yellow in midgut extracts of larvae and adults of *A. l. proxima* and of larvae of *E. vigintioctopunctata*, indicating that lipase was present. In the case, however, of adults of *Epilachna*, any change in colour was difficult to detect, suggesting that only traces of lipase were present in the midgut of adults of *E. vigintioctopunctata*.

The presence of lipase was also investigated by using condensed milk instead of olive oil-acacia gum emulsion. Two drops of bromothymol blue were added to 25 cc. of a 10 per cent. solution of condensed milk and powdered sodium bicarbonate or 1 per cent. sodium hydroxide solution was added until the solution turned light blue. One cc. of blue milk solution and 2 drops of the extract were placed in a microtube and the rest of the tube filled with toluene as usual and incubated for 24-48 hours at room temperature. After 48 hours, the colour of the blue milk changed to yellow in the extracts which reacted in the olive oil-acacia gum test thus confirming the presence of lipase.

Protease.

The white of a hen's egg was placed in a test tube and drawn up in fine capillary tubing; the two ends of the tube were sealed by heating in a flame and then it was transferred to a water bath and heated slowly to coagulate the albumen without air bubbles. The tubing, on cooling, was cut into lengths of about 1 cm. and a piece immersed in gut extract. The pH of the extracts had been adjusted to 8.0 or 6.4 to make the medium alkaline or acidic, respectively, by suitable buffer solutions. The digestion of albumen was readily visible in the test microtube of only the midgut extract of the two insects, and in acidic media only, suggesting that protease is present in the midgut extracts.

Discussion.

Feeding habits.

Undoubtedly certain species of insects are polyphagous and do not appear to be governed in their choice of food by a sense of taste; but as Trager (1947) has pointed out, most species of insects are restricted to certain food-plants. The food-selection mechanism of the more highly specific forms is an effective one, but it cannot serve as a guide to the study of the fundamental nutrients required, because food selection in most insects is determined by characters of the food which probably play no part in the fundamental nutrition of the insect (Trager, 1947). The present study indicates that there are three major factors, all or some of which influence the selection of food, *i.e.*, odour, taste and age of the food-plant. As the larvae and adults of *A. l. proxima* and *E. vigintioctopunctata* are restricted to cruciferous and solanaceous plants, respectively, it is evident that they exercise their sense of smell in the matter of selection of food. The preference of *A. l. proxima* for turnip leaves and of *E. vigintioctopunctata* for brinjal leaves goes to show that they exercise their sense of taste as well. It is concluded that in the particular field observation quoted on p. 289, the factor responsible for the occurrence of *A. l. proxima* on the mustard plants and not on other cruciferous plants, including turnip, growing side by side, was age and not taste, since it was noted that the mustard plants were much younger than the other cruciferous

plants growing nearby. *E. vigintioctopunctata* does not show any special preference for younger plants of brinjal even when young and old plants are grown side by side.

Hydrogen-ion concentration.

In insects in general the pH of the various parts of the gut, particularly the midgut, is never strongly acidic or strongly alkaline, it usually varies only slightly from neutrality. It is only in some exceptional cases that deviation from this general rule is known to occur, *e.g.*, in blowfly larvae and adults and in housefly larvae (Hobson, 1931; Waterhouse, 1940), in Aphids (Bramstedt, 1948) and in adult mosquitos (MacGregor, 1931), and there are cases where pH of the gut of the larvae of Lepidoptera and Trichoptera (Shinoda, 1930*a,b*) varies from weakly alkaline to strongly alkaline.

There is a great deal of controversy as to whether the pH of food affects the pH of the alimentary canal or not. Swingle (1931), after experimenting on larvae of *Popillia japonica* Newm. from 92 samples of soil varying in pH from 6 to 8, and noting the influence of different soils on the pH of the gut, came to the conclusion that the pH of the soil in which the larvae were bred did not affect the pH of the gut and the mixture within the digestive tract had a fairly constant pH. Crozier (1924) has worked on the pH of different parts of the alimentary canal of transparent aquatic larvae and Bishop (1923) on the pH of blood in the larvae and pupae of honey bees. Jameson & Atkins (1921) have worked on the pH of blood and of the alimentary canal of the silkworm, and Bodine (1925) on the pH of grasshoppers. Bodine measured the pH after clearing the alimentary canal of its food contents by cutting it open lengthwise or by starving the insects for various periods. He has recorded that the pH of the starved individuals was similar to that found in normal individuals.

In the present work it has been shown that the pH of different food-plants of *A. l. proxima* varies from 6.8 to 7.2 whereas the pH of different parts of the gut of the larva and adult varies from 6.4 to 7 and from 6.4 to 6.6, respectively. The pH of the leaves of different food-plants of *E. vigintioctopunctata* varies from 6.4 to 7.0, whereas that of the gut of the larva and adult varies from 6.0 to 6.8 and from 5.4 to 6.2, respectively. In neither insect, therefore, are the hydrogen-ion concentrations of the food and of the alimentary canal identical, but, for the reasons given below, no firm conclusion on the influence of the one upon the other can be derived from this observation.

It may be repeated, however, that Swingle (1931) found that the pH of food did not influence the pH of the gut. This is reasonable, in view of the fact that a particular enzyme acts at a particular pH, for, were the pH of the gut to vary with any change of food-plant, it would be likely to disturb the action of the enzymes, leading finally to disturbance in digestion and subsequently the nutrition and health of the insect.

The food-plants of each of the two insects studied here belong to a particular Natural Order, those of *Athalia* to the Cruciferae and of *Epilachna* to the Solanaceae. Naturally, it is to be expected that there will not be much difference in the pH of the different food-plants within each Natural Order. Indeed, a study of species with polyphagous feeding habits, where the insects feed regularly on the leaves of food-plants of various Natural Orders, is likely to throw more light on this aspect.

The effect of starvation on the pH of the gut of the insect is also a controversial issue. Bodine (1925) has noted that the pH values of starved insects were similar to those of normal ones. The author feels that the insect should be partially starved, to clear the gut of the food to a very great extent, before noting the pH of the gut, but that total starvation is likely to affect the pH of the alimentary canal.

The author's observations are in agreement with those of Wigglesworth (1950), Waterhouse (1949) and others that the pH of insect gut is usually only weakly acid or near to neutrality.

Enzymes.

The qualitative test for enzymes has revealed that the salivary glands of the larva and adult of *Athalia* contain only amylase. The salivary glands of the larva of *Epilachna* also contain amylase but those of the adult contain none, although it is rather unexpected that amylase should be present in the salivary glands of the larva of *Epilachna* but not of the adult, which has similar feeding habits. The presence of amylase or of no enzymes at all in the secretion of the salivary glands in these insects is in agreement with the statement of Wigglesworth (1950). There are two possible explanations for the absence of amylase from the salivary glands of adult *Epilachna*; either the adult utilises no starch, or the secretion of amylase from the midgut is enough to digest sufficient starch for its requirements. As the presence of amylase has been noted in the midgut of the adult, the second probability appears more reasonable.

The absence of digestive enzymes in the fore- and hindgut, noted in the present investigations, is also in agreement with the statements of Wigglesworth (1950).

The presence of amylase, maltase, invertase and lactase indicates that these insects utilise carbohydrates, and the presence of lipase suggests the need of fat for their nutrition. Traces only of lipase appear to be present in adults of *Epilachna*, for a positive result was obtained only after keeping the microtube standing for 3 to 4 days, whereas with the larva and the adult of *Athalia* and the larva of *Epilachna* a positive result was obtained in about 24 to 30 hours. Protease is present in the two insects, both in the larvae and adults. The presence of these enzymes leads us to conclude that these insects are able to digest starch, maltose, glucose, lactose and proteins and that these substances are necessary for their nutrition.

If these conclusions are compared with the results published by previous workers, it appears that different phytophagous insects differ in their ability to digest the various constituents of the food eaten. Thus, Ullmann (1932) has demonstrated that the intestinal juices of a number of phytophagous insects are unable to digest the starch granules but can quickly hydrolyse boiled soluble starch. Crowell (1943) has also noted that starch is not digested in Lepidoptera. Brown (1937) has noted complete absence of protein from the excreta of a grasshopper, showing thereby that it has been utilised by the insect. Bramstedt (1948) and Hinman (1933) also have reached the conclusion that, in the leaf-eating insects, protein is the chief dietary factor and is completely utilised along with soluble sugars.

Summary.

Athalia lugens subsp. *proxima* (Klug) is a pest of cruciferous crops, and shows special preference for turnip. In the matter of selection of food, smell and taste of the food and the age of the plant are important factors. *Epilachna vigintioctopunctata* (F.) is a pest of solanaceous plants, particularly brinjal. In this insect only smell and taste are important factors in the selection of food.

The hydrogen-ion concentrations of the salivary gland, foregut, midgut and hindgut of the larva of *Athalia* are 6.4-6.6, 6.4-6.8, 6.6-6.8 and 7.0 and of the adults 6.2-6.4, 6.4-6.6, 6.4-6.6 and 6.6, respectively. The hydrogen-ion concentration of the salivary gland, foregut, midgut and hindgut of the larvae of *Epilachna* are 5.4, 6.4-6.8, 6.0 and 6.0 and of the adult 6.6-6.8, 6.2, 6.0 and 5.4-5.7, respectively.

The foregut and hindgut of the larvae and adults of *Athalia* and *Epilachna* do

not secrete enzymes; the salivary glands of both larva and adult of *Athalia* secrete amylase, the midgut epithelium of both larva and adult of *Athalia* secretes amylase, maltase, invertase, lactase, lipase and protease. The salivary glands of the larva of *Epilachna* secrete amylase, but those of the adult do not, and the midgut epithelium of both larva and adult secretes amylase, maltase, invertase, lactase and protease. The midgut of both larva and adult of *Epilachna* secretes lipase also, although in the adult no more than traces are detectable. The proteases in both insects act in slightly acidic media.

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EXPERIMENTS WITH A SYSTEMIC INSECTICIDE FOR THE CONTROL OF *SCHOENOBIOUS INCERTULAS* (WLK.) (LEPIDOPTERA, PYRALIDAE), A STEM BORER OF PADDY IN WEST BENGAL.

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The introduction of systemic insecticides, which are absorbed into the plant and translocated to all parts of it through its conducting system, offers a new approach to the control of certain classes of insect pests. The present investigation was undertaken to test the possibility of controlling the Paddy Stem Borer, *Schoenobius incertulas* (Wlk.), with "Tetrax" 1, a product containing technical schradan equivalent to approximately 42 per cent. octamethyl pyrophosphoramidate + approximately 25 per cent. triphosphoric acid pentadimethylamide.

Iyatomi (1951) carried out experiments in Japan to determine the comparative efficacy of Folidol-E605, the active principle of which is parathion, and Systox, of which the active principle is demeton (diethyl 2-(ethylmercapto)ethyl thiophosphate), against Rice Stem Borer, and found Folidol-E605 to be the superior. Nagaraja Rao & Krishnaswamy (1952) in India experimented with the different systemic insecticides against different crop pests. They obtained an appreciable reduction in the population of the Rice Mealybug, *Ripersia oryzae* Green, by using schradan, but found that a concentration of 0.75 per cent. had to be used to give a percentage mortality of 52.2 in 72 hours.

Material and Method.

The 1953 experiment.

In the year 1953 a field trial was laid out at the Agricultural Farm, Bankura, to find out how far "Tetrax" 1 could be effectively utilised in combating *S. incertulas*. A plot measuring 47 ft. \times 20 ft. was broadcast with 1½ lb. of paddy seed (variety Ajan 246) previously soaked in 227 cc. of 0.1 per cent. water solution of "Tetrax" 1, and a control plot measuring 47 ft. \times 20 ft. was broadcast with the same quantity of untreated seed. Both the treated and control plots were left without further treatment and when the grain ripened the extent of damage done by *S. incertulas* was recorded by counting the white ear heads and healthy ear heads. The results did not show any significant difference between the two plots, although the percentage of damage was a little higher in the control. Both the plots were harvested and the yields on the treated and control plots were observed to be 62 lb. and 45 lb., respectively (an increase of 37.8%).

The 1954 experiment.

A second experiment was laid out at the same Farm at Bankura in 1954 to obtain further information. The variety Ajan 246 was again used. Two lb. of seed were soaked in 300 cc. of water solution containing 0.1 per cent. "Tetrax" 1 for eight hours. The seed was thoroughly washed in water and sown in a seed bed on 16th June 1954. Another seed bed was sown on the same day, without any chemical treatment, to act as a control. Seedlings from these two seed beds were then transferred to four experimental plots, two as treated plots and two as control plots, on 23rd August 1954. Each plot was 32 ft. \times 32 ft. and the total area of the experiment was 66 ft. \times 66 ft. or 0.1 acre. The treated and control plots were arranged at random.

TABLE I.
The effect on yield of treating paddy with "Tetrax" I.

Plot no.	Treatment	No. of paddy plants	Total plants	No. of ear heads	Total ear heads	Difference between A and B	Yield in lb.	Total yield in lb.	Difference between A and B in lb.
I	Tetrax I } A	1412	2876	19476	39372	4845	52.98	108.68	31.4
III	Tetrax I	1464		20496			55.70		
II	Control } B	1332	2942	14519	35127		29.29	77.27	
IV	Control	1610		20608			47.98		

TABLE II.
The effect on the control of *Schoenobius incertulas* of treating paddy with "Tetrax" I.

Plot no.	Treatment	Average ear heads per plant	No. of ear heads	Ear heads damaged per plot	Total damaged per treatment	Per cent. damage	
						Per plot	Per treatment
I	Tetrax I } A	13.8	19476	71	127	.36	.32
III	Tetrax I	14.0	20496	56		.27	
II	Control } B	10.9	14519	79	141	.54	.40
IV	Control	12.8	20608	62		.30	

The paddy seedlings of the treated plots were further subjected to two treatments; before transplanting they were dipped in "Tetrax" 1 and later, after a lapse of about three weeks, the established plants were sprayed with 0.1 per cent. water solution of the same insecticide.

The paddy seedlings to be transplanted were steeped in 0.1 per cent. "Tetrax" 1 solution for eight hours on 22nd August 1954 and then washed thoroughly in clean water. Two plots were planted with these treated seedlings on the 23rd August 1954. The weather on that day was: maximum temperature 84°F., minimum temperature 78°F., humidity 68 per cent., rainfall 0.01 in. and cloud 2/3. There was no rainfall within 24 hours of treatment.

The spraying treatment was applied on 14th September 1954 when the conditions were: maximum temperature 84°F., minimum temperature 79°F., humidity 73 per cent., rainfall 0.18 in. and cloud 2/5. No rainfall was noted within 24 hours of treatment.

All the plots were harvested on 2nd December 1954 and data from the experiment are given in Tables I and II.

The general climatic conditions during the period of the experiment at Bankura are shown in Table III.

The results of the experiment have also been represented by histograms in fig. 1, for a visual comparison.

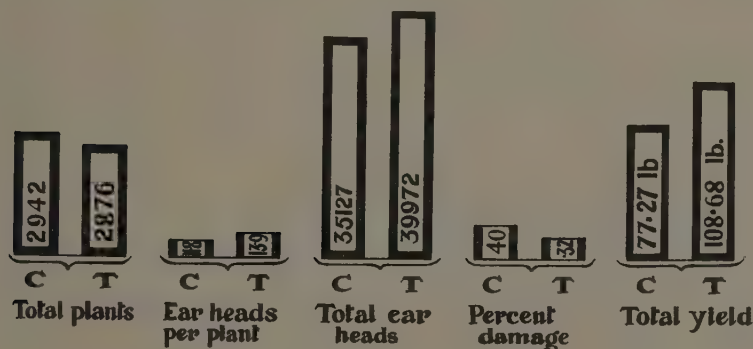


Fig. 1.—Results of the 1954 experiment (C = control; T = treated).

Discussion.

An examination of Table I and fig. 1 reveals that the difference in the total number of plants in the treated and control plots is not significant; indeed the control plots produced slightly more plants. But on the other hand it will be seen that the treated plots produced 4,845 ear heads more than the control plots. The result of this increase in number has a direct bearing on the yield, the treated plots producing 31.4 lb. more paddy than the control plots (an increase of 40.5%). This increase in yield cannot be attributed to the lower incidence of *S. incertulas* in the treated plots because it will be seen from Table II that the number of damaged ear heads was only 14 more in the control than in the treated plots. It seems evident that treatment with the systemic insecticide gave rise to an increase in the production of ear heads and consequently to a higher yield. A similar increase in yield had also been noted in the results of the 1953 experiment.

Unfortunately the incidence of *S. incertulas* in both the years was too low for any definite conclusion on control to be drawn. It would be interesting to find what effect similar treatment has in years when the incidence of the pest is heavy

TABLE III.
The general weather conditions during the experiment.

Year and month	Average temperature of the month in °F.		Average relative humidity of the month (%)	Rainfall	
	Max.	Min.		No. of days	Total (in.)
1954					
June	94.4	78.3	67.3	10	6.06
July	91.6	78.3	71.3	15	8.74
Aug.	89.4	78.2	72.6	15	9.71
Sept.	89.2	80.2	76.5	20	10.50
Oct.	87.2	70.9	64.8	6	3.41
Nov.	83.2	57.6	63.2	nil	nil

and whether an increase in yield in treated over untreated plots is shown under such conditions.

Summary.

Experiments were carried out in West Bengal to ascertain whether the Paddy Stem Borer, *Schoenobius incertulas* (Wlk.) could be controlled by "Tetrax" 1, a systemic insecticide containing technical schradan equivalent to approximately 42 per cent. octamethyl pyrophosphoramidate + approximately 25 per cent. triphosphoric acid pentadimethylamide.

In a preliminary experiment, treatment had little effect on the damage caused by the borer but the yield of grain was higher from a treated plot than from a control plot.

A further experiment was carried out, the following year, in which the seed was soaked in a 0.1 per cent. water solution of "Tetrax" 1 for eight hours before sowing in the seed beds. The seedlings were lifted about 2½ months later and steeped in 0.1 per cent. "Tetrax" 1 for eight hours before transplanting into two plots, each 32 ft. × 32 ft. About three weeks later the plants were sprayed with a solution of the same strength. Two plots of similar size, in which the paddy had received no treatment, were maintained as controls.

Again there was very little difference, between the treated and untreated plots, in the damage caused to the ear heads by the pest, but the treated plots produced 108.68 lb. of grain as against 77.27 lb. for the untreated plots. The increase was largely due to an increase in the number of ear heads per plant.

The incidence of the borer was too low in both experiments to determine whether "Tetrax" 1 exercised any appreciable control, but the treatment did give rise to a higher yield of grain under the conditions of the experiments.

References.

- IYATOMI, K. (1951). Pamphl. Shizuoka agric. Exp. Sta. November, 1951.
NAGARAJA RAO, K. R. & KRISHNASWAMY, S. (1952). Plant Prot. Bull., New Delhi, 4, pp. 56-62.
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THE PERIODICITY OF APHID FLIGHT IN EAST AFRICA.

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The importance of an Aphid as a virus vector depends on factors additional to its ability to transmit a virus from an infected to an adjacent healthy plant. The number of flying Aphids may determine the initial Aphid population and in the case of a virus vector the initial virus infection. There are a number of published records of the seasonal periodicity of Aphid flight in England (Broadbent, 1946; Broadbent & Doncaster, 1949; Doncaster & Gregory, 1948; Johnson & Eastop, 1951; Thomas & Vevei, 1940). In Europe, the flight of many Aphids is concerned with their migration, from primary to secondary hosts in the spring and in the reverse direction in the autumn, in addition to summer "distribution" flights. In East Africa, alternation between primary and secondary hosts is unknown, and all Aphid flight apparently corresponds to the summer flights in Europe.

Continuous trapping has been carried out at Muguga, near Kikuyu, Kenya, for more than two years. The headquarters of the East African Agriculture & Forestry Research Organisation at Muguga is 6,850 ft. above mean sea level, and is situated in a cleared area in a planted wattle forest, surrounded by forest and African cultivation. The average rainfall of 36 in. falls mainly in April and May (long rains, ± 17 in.) and during October to December (short rains, ± 8 in.).

The data available are (1) from three Moericke trays (shallow, yellow-painted, water-filled trays), from mid-December 1952 to January 1955, and (2) from a suction trap of the type described by Johnson (1950) from mid-January 1953 to January 1955. Yellow trays were also operated at Nachingwea, Southern Province, Tanganyika, from November 1953 to March 1954. Nachingwea (one of the Overseas Food Corporation's areas) is about 1,200 ft. a.m.s.l. and had a rainfall of 24 in. from December 1953 to mid-March 1954 (Nov., 0.20"; Dec., 5.28"; Jan., 9.09"; Feb., 6.68"; 1-18 March, 2.87"). The vegetation around the planted area is *Brachystegia-Isobерlinia* woodland. The yellow trays were mounted on posts or other stands and stood about 2 ft. 6 in. above ground level. The mouth of the suction trap was about 3 ft. 6 in. above ground level. At Muguga, all the traps were placed in a small plot of pyrethrum not infested with Aphids. At Nachingwea, 12 trays were scattered in different situations.

Seasonal Periodicity.

Details of the catches at Muguga and Nachingwea are set out in Tables I and II. Nomenclatorial and biological data for the Aphids concerned are given elsewhere (Eastop, in press). The detailed relation of trap catches to Aphid population on the plant, percentage alatae, moulting rhythm and weather conditions is not attempted here. Johnson (1954) has discussed these problems and gives a bibliography of about 60 references. It is not possible to relate the East African data to weather conditions, as this would have required trapping and meteorological data from different heights above ground level, which was not possible in this work. However, the data given here do show the great differences in numbers of Aphids in the air at different seasons. The considerable agreement

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TABLE I.

Aphids from yellow trays (roman) and suction trap (italics), Muguga, Kenya, 1959-1954.

	1953												1954													
	J	F	M	A	My	Ju	Jy	Au	S	O	N	D	J	F	M	A	My	Ju	Jy	Au	S	O	N	D	Total	
<i>Acyrtosiphon pisum</i> (Harris)	..	0	0	0	0	4	25	19	0	0	1	1	2	1	0	0	2	47	115	11	1	0	0	0	0	230
<i>Aphis nerii</i> Boy.	..	0	0	0	0	6	43	24	0	0	0	0	1	1	0	0	2	41	135	17	0	0	0	0	0	284
<i>Aphis</i> spp.	..	0	0	0	0	0	10	1	0	0	0	0	0	0	0	0	1	3	39	7	0	0	0	0	0	63
<i>Aphis</i> spp.	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
<i>Aponeura lentis</i> (Pass.)	..	0	2	6	18	393	38	14	0	3	9	10	72	28	31	12	3	61	764	189	5	4	1	3	4	1694
<i>Aponeura lentis</i> (Pass.)	..	10	18	0	1	0	16	7	3	5	4	2	10	4	4	4	1	3	107	26	2	6	0	0	0	353
<i>Aulacorthum solani</i> (Kalt.)	..	0	0	0	0	1	6	4	2	2	0	0	0	0	0	0	0	1	23	7	4	1	0	0	102	
<i>Brevicoryne brassicae</i> (L.)	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	7	9	2	0	0	33	
<i>Brevicoryne brassicae</i> (L.)	..	0	6	5	8	1	0	54	15	37	60	52	24	9	9	0	1	3	10	8	4	3	0	0	12	833
<i>Diuraphis compositae</i> (Theo.)	..	0	1	0	0	0	7	3	13	2	0	0	1	1	11	13	5	7	44	17	3	3	0	0	0	210
<i>Hyadaphis coriandri</i> (Das)	..	1	3	1	0	6	28	8	9	0	0	2	2	6	2	2	1	5	4	0	0	0	0	0	0	96
<i>Hysteronura eiperi</i> (v.d. Goot)	..	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	
<i>Lepaphis pseudobrassicae</i> (Davis)	..	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	55	61	0	0	0	0	0	123
<i>Longitarsus sacchari</i> (Zhm.)	..	11	0	0	0	2	275	16	2	0	0	1	6	15	2	5	0	4	92	112	3	0	0	0	0	221
<i>Macrosiphum euphorbiae</i> (Thos.)	..	0	2	0	0	0	4	0	0	0	10	1	4	2	1	0	0	9	116	50	1	7	2	1	10	526
<i>Macrosiphum (Sitobion) spp.</i>	..	0	0	0	0	0	14	1	0	0	0	0	0	0	0	0	0	13	4	0	0	0	0	0	0	58
<i>Macrosiphum (Sitobion) spp.</i>	..	0	0	0	0	0	14	1	0	0	0	0	0	0	0	0	0	18	8	0	0	0	0	0	0	31
<i>Metromyzus mitegoni</i> Eastop	..	0	1	1	2	12	9	3	1	1	0	0	0	0	0	0	0	5	30	3	0	0	0	0	0	9
<i>Myzus ornatus</i> Laing	..	0	0	0	0	0	8	0	1	1	0	0	0	0	0	0	0	31	12	4	1	0	0	1	1	60
<i>Myzus persicae</i> (Sulz.)	..	5	3	0	0	3	235	16	0	0	0	0	0	0	0	0	0	328	150	5	1	0	0	0	0	826
<i>Rhopalosiphum maidis</i> (Fitch) ♀♀	..	0	6	0	1	2	52	50	38	19	2	2	11	10	6	6	9	138	73	8	2	0	0	0	0	128
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	4	3	0	3	10	114	79	26	3	3	6	8	7	3	4	10	138	73	8	2	0	0	0	149
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44	31	8	9	1	0	0	0	147
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	8	0	0	0	0	0	0	9
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhopalosiphum maidis</i> (Fitch) ♂♂	..	0	0	0																						

* In January 1953 the suction trap ran only from 17th to the end of the month.

† In "Aphis" spp., were mostly *A. fabae solanella* Theo. and a few *A. craccivora* Koch.

"Other" spp., comprises *Geotica lutea* (Zhm.), 19, 3 (yellow-tray catch in roman, suction-trap catch in italics) and *Smythurodes betae* Westw. (*Triidaphis* "phosoli" Pass.), 19, 2.

"Other Aphids ♀♀" comprises *Acyrtosiphon bidentis* Eastop, 4, 5; *Altophysa mureti* Essig, 1; *Aphis* (*Brachyaphis*) *pseudocircoides* (Theo.), 1; *Brachyaphis* *amygdalini* (Schout.), 1, 1; *B. heliophila* (Kalt.), 19, 2; *Capitophorus hippocrepae mitegion* Eastop, 15; *Glaucosiphon frugifoli* (Ckll.), 3, 9; *Olethoragus* (Wlk.), 1, 1; *Eucarazia elegans* (Ferrari), 2; *Hyalosiphum* (*Schizaphis*) *graminum* (Rondt.), 11, 7; *Macrosiphoniella samborni* (Gill.), 4, 5; *Macrosiphum rosae* (L.), 1, 2; *Microgaster ageni* Eastop, 19, 16; *Nasonovia* (*Hyperomyzus*) *lactuca* (L.), 20, 7; *Neophylaphis* *grobleri* Eastop, 4; *Neotoxoptera violae* (Perk.), 1, 2; *Rhopalosiphoninus atropurpureus* (L.), 1; *Rhopalosiphum* *nymphalae* (L.), 1; *Rh. podi* (L.), 3, 15; *Saltusaphis* *acirpa* Theop., 4; *Sappaphis* *phosoli* Pass., 19, 2.

between results from the suction trap and the yellow trays lends confidence to the conclusions to be drawn from them.

The general picture for 1953 and 1954 at Muguga is similar, for both yellow trays and suction trap. About half the year's catch was taken in June and about one quarter in July, leaving only one quarter of the annual catch for the other ten months. The catch continued to decrease from August onwards and through the short rains, but began to increase in December or January. It fell off again from February to April. This pattern is a reflection of the large increase in Aphid population on young plant growth that is to be observed at the end of and after

TABLE II.

Aphids from yellow trays, Nachingwea, Tanganyika.

	1953			1954						
	Nov.		Dec.	Jan.		Feb.		Mar.		Total
	16-30	1-15	16-31	1-15	16-31	1-14	15-28	1-14	15-25	
<i>Aphis craccivora</i> Koch	1	0	0	0	0	8	11	9	1	30
" <i>gossypii</i> Glov.	0	0	0	0	0	7	32	32	20	91
" <i>nerii</i> Boy.	1	0	0	0	0	0	0	1	0	2
<i>Brachyunguis evansi</i> Eastop ..	2	0	0	0	2	2	0	0	0	6
<i>Dactynotus compositae</i> (Theo.) ..	0	0	0	0	0	1	2	3	5	11
<i>Longiunguis sacchari</i> (Zhnt.) ..	0	0	0	0	18	92	634	41	2	787
<i>Macrosiphum (Sitobion)</i> sp. ..	0	0	0	0	0	0	0	1	1	2
<i>Pentalonia nigronervosa</i> Coq. ..	0	0	0	0	1	0	0	0	0	1
<i>Pterasthenia shiraensis</i> Stroyan ..	0	0	0	1	0	0	1	0	0	2
? <i>Pterasthenia</i> sp.	0	0	0	0	0	0	1	0	0	1
<i>Rhopalosiphum maidis</i> (Fitch) ..	0	0	0	0	0	1	41	7	5	54
" <i>ruftabdominalis</i> (Sasaki) ..	0	0	0	0	0	5	1	0	1	7
<i>Schoutedenia bougainvilleae</i> (Theo.) ..	0	0	7	54	44	72	22	5	4	208
<i>Tetraneura hirsuta</i> (Baker) ..	0	0	0	0	0	0	7	10	19	36
<i>Unipterus commiphorae</i> Doncaster ..	0	0	0	0	0	1	3	3	1	8
" <i>nachensis</i> Eastop	0	0	0	1	5	12	3	0	3	24
" spp.	0	0	0	0	4	12	66	6	3	91
Total	4	0	7	56	74	214	828	118	55	1361
Rainfall (in.)	0.0	1.9	3.4	4.7	4.4	1.5	5.2	2.9	—	24.1

The *Unipterus* spp. were mostly *U. ayari* Eastop with 6 specimens of *U. ufuasi* Eastop, 1 of *U. sarcus* Eastop and two males of *U. commiphorae persimilis* Eastop. All the examples of *U. commiphorae* were of the subspecies *persimilis*.

the rains, and which is followed by a rapid build-up of predators. The maximum catch of most species took place in June or July but there were a few exceptions. *Brevicoryne brassicae* (L.) occurred in large numbers in September and October, when most other species were present in small numbers only. It may be noted that in England *B. brassicae* also flies later than most other species. *Hyadaphis coriandri* (Das), which is closely related to *Brevicoryne*, although caught in only small numbers, apparently also had a later peak of abundance than most other Aphids. The root-feeding Fordini (*Aploneura*, *Geoica* and *Smynthuroides*) had a greater proportion of their annual catch in January and February than most other species. *Tetraneura hirsuta* (Baker), another grass-root feeding species, was intermediate between the Fordini and the other Aphids in its times of flight. The suction-trap data are more reliable for the calculation of relative seasonal abundance since the numbers caught by the yellow trays depend to some extent on the intensity with which these are illuminated by the sun.

At Nachingwea, Tanganyika, the trap catches (Table II) were related to the rains in a similar way to the catches at Muguga, Kenya. The list of Aphids trapped at Nachingwea is typical of the drier areas of East Africa, *i.e.*, few species and many of these associated with grasses, Combretaceae, Burseraceae, Asclepiadaceae and Euphorbiaceae.

Diurnal Periodicity of Flight.

The successive hourly catches in the suction trap (Table III) at Muguga show no such distinct double peak of aerial density as has been shown for *Aphis fabae* Scop. (Johnson, 1949) and some other Aphids (Eastop, 1951), in Europe. One reason for this may be that the trap was not situated near a large source of Aphids; a double peak of aerial density, related to moulting, would be obscured if a large proportion of the catch came from some distance away and took several hours for the journey. If, however, the double peak is related to flight behaviour, its manifestation would be unaffected by the distance of the source of the Aphids from the traps. Another factor tending to obscure a double peak is the lumping of several days' data, necessitated by the low aerial density of Aphids at Muguga.

Although there are no distinct double peaks, in *Aphis* spp. and *Lipaphis pseudobrassicae* (Davis) there is a sharp rise to a minor peak at 8-9 a.m., followed by a drop and a gradual rise to 14-15 hr. In the catch of *Rhopalosiphum maidis* (Fitch) there is also a sharp rise at 8-9 a.m., but without a subsequent drop. Instead, there is a gradual and fairly steady rise to 15-16 hr.

The catches for the other species, though small in numbers, tend in most cases to show a similar small peak between 8 and 9 a.m.

This small but fairly constant increase in catch at that time may correspond to the morning peak in Europe.

Selection of Species by the Yellow Trays.

Some Aphids are more strongly attracted than others by yellow (Eastop, 1955), grass- and sedge-feeding species being only weakly attracted as compared with most dicotyledon-feeding species. Moericke (1955) has published some data, comparing suction traps and yellow trays in Europe, in which concurrent catches of *Myzus persicae* (Sulz.) and (in brackets) *Rhopalosiphum padi* (L.), a grass-feeding species, were 96 (69) in a suction trap and 256 (32) in a yellow tray. Comparable data for these species from the present work in Kenya are 128 (13) in a suction trap and 826 (3) in yellow trays. The attraction of *M. persicae* by the yellow trays is very marked.

Summary.

Continuous trapping of winged Aphids was carried out with Moericke trays and a suction trap for two years at a site at 6,850 ft. above sea level in Kenya, and with Moericke trays, for four months only, at a site at 1,200 ft. in southern Tanganyika.

The trap data show a marked seasonal periodicity of flight (three-quarters of the annual catch in two months) at the Kenya site. The large catch is a reflection of a large increase in population following the rains. A similar relation between size of catch and the period of the rains is indicated by the data from Tanganyika.

Successive hourly catches with a suction trap show no distinct diurnal double peak of aerial density such as occurs in Europe.

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METHOD FOR BREEDING, HANDLING AND SEXING ADULTS OF *DROSOPHILA MELANOGASTER* MG. AS A TEST INSECT FOR BIOASSAY.

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(PLATE VII.)

Apart from its use for genetic purposes, *Drosophila melanogaster* Mg. has become increasingly important as a laboratory test insect in methods used for the determination of microquantities of insecticides.

Such microbioassay techniques, however, require special means for standardisation. The purpose of the work described below was to improve the efficiency of the breeding technique for *D. melanogaster* and to improve the standardisation of the conditions under which the insects are bred and selected according to age.

Because males have been found to be about twice as susceptible as females, it is advisable for microbioassay tests to use males only. An apparatus for efficient separating of the sexes was specially designed for this purpose.

Breeding Method.

In order to meet the necessary requirements for a regular supply of adults of *Drosophila* for microbioassay purposes, a simple method has been developed which:

1. enables large quantities of adults of *Drosophila* to be obtained in a relatively short time, bred under standardised conditions;
2. provides a quick separation of the one-day-old flies from the breeding jars;
3. enables sexing to be performed in an efficient manner.

Breeding is done in an air-conditioned cupboard at a temperature of 24 to 25°C. and a relative humidity of between 60 and 80 per cent. The nutrient medium consists of the following ingredients:

Maize flour	250	gm.
Sugar	125	gm.
Brewer's yeast	15.5	gm.
Agar agar powder	12.5	gm.
10% Nipagin M* solution in ethanol 96%	10	ml.
Water	1000	ml.

After dissolving the agar powder in hot water, a mixture of the other dry ingredients is added and heated for about five minutes. Finally the Nipagin solution is added to the medium which is then poured into four 1-lb., wide-mouth jars. After cooling, 1 ml. of a 50 per cent. baker's yeast suspension is applied to the surface of the medium in each bottle. Having introduced the initial population, each breeding jar is closed with a piece of muslin fixed over the top by means of a rubber band.

* Methyl ester of p-hydroxybenzoic acid, to prevent excessive moulding.

Influence of Physical Conditions of the Culture Medium.

It was found that the physical conditions in the culture jars exerted an appreciable influence on the breeding. An experiment was carried out using the above-mentioned nutrient medium under three different conditions:

- (a) surface of the medium flat and unbroken, with a wad of cotton-wool laid on it to provide suitable sites for pupation;
- (b) same as (a) but with a layer of cotton-wool 1 cm. thick under the medium in order to absorb excess of liquid;
- (c) same as (b) but nutrient medium broken into irregular lumps, and a vertical hole made in the centre which was filled with cotton-wool.

The difference between (a) and (b) mainly refers to moisture conditions, whereas that between (b) and (c) concerns better aeration and increased surface area.

Starting from 50 females for method (a) and 20 females for methods (b) and (c), together with an equal number of males, the rates of breeding proved to be widely different. A summary of the results obtained from the breeding experiment is given in Table I. Attention was paid to the number of days between introduction of the initial population to the culture and the first emergence of adults, to the day of maximum emergence, to the variation in sex ratio (males to females), to the yield in number of flies per culture jar, and to the progeny per female.

TABLE I.

Influence of physical conditions of the culture medium on the breeding of
Drosophila melanogaster.

	Method (a)	Method (b)	Method (c)
Number of days between start of culture and first emergence ..	12 to 13	11 to 12	11
Day of maximum emergence ..	6th to 7th	4th	3rd
Variation in sex ratio	0.4 to 1.1	0.46 to 1.31	0.93 to 1.15
Yield per culture jar (number of flies)	ca. 2500	ca. 3000	ca. 4000
Progeny per female	ca. 50	ca. 160	ca. 200

As is obvious from the above figures, the breeding method mentioned under (c) offered the best advantages, viz., an earlier commencement of the emergence period after the culture has been started, a quicker maximum emergence, a more constant sex ratio (near to the value 1), a higher progeny per female and, consequently, a higher yield of flies per culture jar.

Procedure to obtain Adults of *Drosophila* of known Age.

The use of *Drosophila* for bioassay experiments demands that particular care should be taken to obtain flies of known age. A simple automatic device has been constructed for the separate collection of all flies emerging in each 24-hour period, which means that the age of each batch of *Drosophila* is accurate within the limit of one day.

The procedure is as follows: When the first new generation is to be expected (about 10 days after the start), the old flies (initial population) are removed from the breeding jars. The open jars (a) (see Pl. VII, fig. 1) are then placed in a light-proof emergence box (b) provided with an opening to which an exit funnel

(c) has been connected. This funnel is blackened except for the tube. The tube ends in a cylindrical 3-litre collecting jar (d) containing a layer of cotton-wool (e) soaked with a solution of sugar in water.

By means of a suction tube (f) a gentle stream of air is led through the collecting jars and the box in order to prevent the former from becoming too wet, and to ensure that possible noxious gases from the fermenting medium do not inhibit the development of the flies.

It appeared that the newly emerged flies are immediately attracted by the light entering through the tube in the funnel. In a very short time they arrive in the collecting jar, in which they can stay for over a week with less than 5 per cent. natural mortality.

A trial with about 1,500 newly emerged flies, placed in the light-proof box, showed that 75 per cent. of the flies arrived in the collecting jar within six hours, and 98.5 per cent. within 24 hours. By daily changing of the collecting jars it is therefore possible to obtain large batches of flies of known age.

In order to have a sufficient number of test flies continuously at hand for experimental purposes, breeding series, of four jars each, can be started twice a week at three- to four-day intervals. Each series of four jars (after the ten-day pre-adult period) is kept in the light-proof emergence box for a period of ten days for producing adults, after which period the culture jars are replaced by new ones. Three boxes are then always in operation, providing a continuous production of 3,500 to 6,500 adults of *Drosophila* daily. The extent of the culture, of course, can be changed at will.

It appeared that the automatic collecting of newly emerged flies gives a considerable saving of time as compared with other methods.

Sexing of the Flies.

Experiments, in which 0- to 4-day-old adults of *Drosophila* were exposed for 48 hours to filter papers impregnated with known amounts of dieldrin, demonstrated a marked difference in susceptibility between the sexes, the males being about twice as susceptible as the females. Moreover, it could be shown that a flatter regression line was obtained with males than with females, indicating that males give a greater response to differences in dosage of toxicant. It could be calculated that the use of an equal mixture of the sexes would result in a slightly S-shaped regression line (see fig. 1).

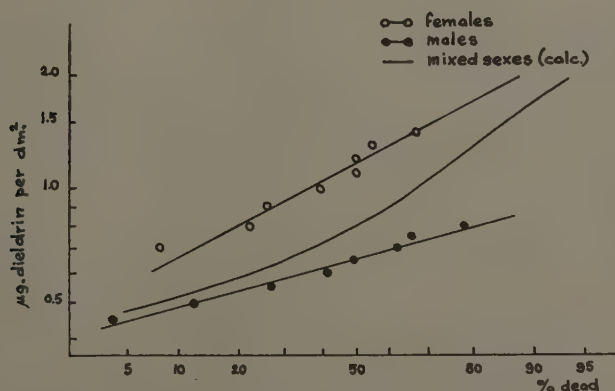


Fig. 1.—Regression lines for males and females of *Drosophila melanogaster* (found) and for an equal mixture of both sexes (calculated).

For these reasons it is advisable for bioassay purposes to use the male sex only. For the purpose of quick and efficient separation of the sexes a special apparatus was designed. This apparatus (Pl. VII, fig. 2) consists of a metal frame (a) with a metal ring (b) on top. This ring has a large cut-out on the right side in order to move an aspirator easily over the whole area. The bottom consists of a Perspex plate (c); the space thus formed is closed with a glass plate. A second ring (d), a little higher and wider, with a small opening (e) for introducing the aspirator tube (f), can be turned around the fixed inner ring. The Perspex plate is provided with an opening in front, leading into a small metal funnel (g), with a side tube to introduce CO_2 as a narcotic. A little conical flask (h), for collecting superfluous flies, is attached to this funnel by means of a rubber stopper. The whole apparatus is fixed at an angle of about 20° . A small lamp (i) serves as light source.

For sexing and counting, the insects are first anaesthetised with CO_2 and then put under the glass plate, where the males are sucked away separately by means of a small aspirator, the tube of which is introduced into the opening in the turnable outer ring. It is possible to turn the outer ring around the inner one by means of the aspirator tube, thus enabling the operator to reach all the flies. The flies are gently passed directly via the aspirator tube, into test tubes, where they recover from the narcosis and are kept ready for the experiment.

A continuous gentle stream of CO_2 /air mixture (3:1) keeps the flies under narcosis during sexing.

It was found that even a much deeper narcosis than is actually given produces no increase in natural mortality nor does it influence the susceptibility of the test flies. Nevertheless, only a slight narcosis was found advisable in order that the test flies might become available for the bioassay as soon as possible.

The extra time involved in separating the sexes, when preparing 100 batches of about 30 flies for bioassay purposes is only about one hour.

Summary.

A method is described by which a continuous supply may be produced, efficiently, of large numbers of adults of *Drosophila melanogaster* Mg. of known age and sex for microbiassay tests.

Equal numbers of male and female flies were introduced into breeding jars containing a nutrient medium and maintained at $24-25^\circ\text{C}$. and 60–80 per cent. relative humidity. They were removed ten days later, just before the emergence of their progeny.

It was found that the physical conditions in the culture jars exerted an appreciable influence on several aspects of the breeding of *Drosophila*. Thus, in trials in which the surface of the nutrient medium was flat and unbroken, with a pad of cotton-wool laid on it to provide suitable sites for pupation, and with or without a layer of cotton-wool beneath it, or in which the medium was broken with irregular lumps, laid on a layer of cotton-wool and arranged with a space, filled with cotton-wool, at the centre, the best results were obtained with the last modification.

Under these conditions, emergence of adults began earlier and reached its peak earlier, a most constant sex-ratio and a higher number of progeny per female, and consequently a higher yield of flies per culture jar, were obtained.

A special device, which takes advantage of the positive phototropism of the flies emerging from a group of culture jars enclosed in a darkened box with an exit funnel leading into a collecting jar, made it possible, by daily replacement of the collecting jar, to remove practically all newly emerged flies within 24 hours, so that the known age of any batch is accurate within the limit of one day.

Male flies have been found to be twice as susceptible as females and give a

greater response to differences in dosage of toxicant. For this reason male flies only are to be preferred for use in bioassay experiments.

Using another device, the flies were therefore sexed, and the required number of batches of males collected. This device consists of a glass-covered container through which a gentle continuous stream of CO_2 /air is passed over the flies, which have already been anaesthetised with CO_2 . The male flies are taken up individually with a small aspirator and passed direct into test tubes where they are collected in batches.



FIG. 1. Light-proof emergence box for adults of *Drosophila melanogaster*.
a, breeding jars; b, light-proof box; c, exit funnel; d, collecting jar; e, layer
of cotton-wool; f, suction tube.



FIG. 2. Apparatus for separating the sexes of *Drosophila melanogaster*.
a, frame; b, inner ring with cut-out; c, "Perspex" bottom plate; d, outer
ring; e, inlet for aspirator tube; f, aspirator tube; g, funnel; h, conical flask;
i, lamp.

EFFECT OF HOST-PLANT ON THE RESISTANCE OF *ACYRTHOSIPHON PISUM* (HARRIS) TO INSECTICIDES.

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A number of factors may influence the resistance to insecticides of a population of a given species of insect of the same age and stage of development. There are indications that the resistance may differ with the host-plant on which the insect is reared. Swingle (1939) gave evidence that the resistance of the 5th-instar larva of the army worm, *Prodenia eridania* (Cram.), to lead arsenate as a stomach poison differed with the host-plant on which it was reared. This was confirmed by Markos & Campbell (1943) using calcium arsenate. Richardson & Casanges (1942) showed that the resistance of the Green Peach Aphid, *Myzus persicae* (Sulz.), to nicotine vapour varied greatly with the host-plant on which the Aphid was reared; insects from nasturtium were very susceptible, those from climbing dahlia intermediate and those from lettuce and turnip most resistant. Yust & Howard (1942) found that California Red Scale, *Aonidiella aurantii* (Mask.), feeding on lemons from different groves sometimes gave markedly different mortalities with HCN fumigation, although the appearance of the fruit was the same. These authors also found that kills on lemons that were slightly soft were better than on those which were firm and turgid.

It seems probable that the differences recorded are due to differences in nutrition; for example, Gaines & Mistic (1952) found that samples of the Boll Weevil, *Anthonomus grandis* Boh., reared from bolls were four times as resistant to toxaphene as those reared from squares. There are also records of differences due to nutrition occurring with insects other than plant-feeding species. Lord (1942) found that, within limits, adults of *Drosophila melanogaster* Mg. increased in resistance to nicotine with increasing amounts of yeast in the rearing medium. McGowan & Gersdorff (1945) found that adults of *Musca domestica* L. which were fed before and after treatment on several different foods differed in their resistance to DDT and pyrethrum. Those fed on fresh skimmed milk with or without formaldehyde were most resistant, those on sugar least resistant. David & Bracey (1946) found that adults of *Aedes aegypti* (L.) fed on water + blood are more resistant to the pyrethrins than those fed on water + sugar.

The work described in this paper resulted from early indications that the resistance of the Pea Aphid, *Acyrtosiphon pisum* (Harris), to rotenone might be different when it was reared on broad bean and when it was reared on clover. The evidence obtained that a difference exists due to host-plant difference, although strong, is not conclusive. Whether or not a difference exists may depend to some extent on whether the dosage is assessed as the amount of poison per individual or the amount of poison per unit weight of insect, since the insects from the different host-plants tended to differ considerably in size.

Experimental Method.

Test species: the Pea Aphid, *Acyrtosiphon pisum* (Harris). Host-plants: broad bean, *Vicia faba*, and red clover, *Trifolium pratense*.

In each experiment the populations of *A. pisum* on each of the two host-plants were derived from a single common parent mother to exclude the possibility of biological races (Harrington, 1943, 1945). The two sets of host-plants were grown side by side in a glasshouse so that the environmental conditions were similar.

TABLE I.
Relative resistance to rotenone of *A. pisum* from broad bean and clover.

Date	Host	w/v % of rotenone to kill			b	x	% Control mortality	Resistance ratio 50% level Clover : Bean	Notes
23.viii.43	Broad bean Clover	25% 0.00010 0.00028	50% 0.00015 0.00037	75% 0.00023 0.00050	3.60 5.04	4.2 [2] not sig. 7.2 [2] sig.	13 3	2.5 sig.	No statement of size or condition.
11.viii.48	Broad bean Clover	0.00011 0.00015	0.00015 0.00021	0.00020 0.00030	5.21 4.92	0.4 [3] not sig. 0.5 [3] not sig.	9 4	1.4 sig.	Bean insects much larger than clover.
24.v.51	Broad bean Clover	0.00020 0.00028	0.00028 0.00043	0.00040 0.00063	4.53 3.84	6.5 [2] sig. 23.1 [3] sig.	25 5	1.5 not sig.	Bean insects large and active. Clover smaller and tended to stick together.
28.vi.51	Broad bean Clover	0.00012 0.00017	0.00016 0.00023	0.00022 0.00031	4.86 5.88	2.5 [2] not sig. 0.1 [2] not sig.	0 10	1.4 sig.	Bean insects very large and active. Clover fairly large and active.
27.v.52	Broad bean Clover	0.00014 0.00017	0.00020 0.00023	0.00028 0.00032	4.61 4.61	1.3 [3] not sig. 3.1 [3] not sig.	5 2	1.2 not sig.	Bean insects all large. Clover mainly medium size, few large.
2.vii.52	Broad bean Clover	0.00015 0.00026	0.00021 0.00032	0.00030 0.00040	4.57 7.59	2.3 [2] not sig. 0.1 [2] not sig.	7 3	1.5 sig.	Both fairly large insects, bean apparently slightly larger than clover.
7.vii.52	Broad bean Clover	0.00028 0.00025	0.00039 0.00036	0.00054 0.00054	4.66 3.95	8.5 [2] sig. 4.8 [2] not sig.	11 31	0.9 not sig.	Bean insects larger than clover, approx. twice as heavy, younger and less crowded. 60 insects weighed, bean 156 mg., clover 78 mg.
30.vi.53	Broad bean Clover	0.00022 0.00020	0.00026 0.00028	0.00031 0.00039	8.82 4.48	3.6 [2] not sig. 3.7 [4] not sig.	0 2	1.1 not sig.	Both active insects, clover slightly smaller.
8.vii.53	Broad bean Clover	0.00016 0.00031	0.00023 0.00042	0.00035 0.00056	3.86 5.09	0.3 [2] not sig. 0.4 [2] sig.	42 7	1.8 sig.	Bean slightly smaller than clover, high control mortality.
21.ii.56	Broad bean Clover	0.00016 0.00025	0.00030 0.00051	0.00055 0.00105	2.53 2.15	1.5 [3] not sig. 0.9 [3] not sig.	13 7	1.7 not sig.	Weight: 50 insects from bean = 117 mg., 50 insects from clover = 81 mg.

No rigid measures were taken to prevent infestation from outside sources but this would not appear likely.

The poison used was pure rotenone (MP. 161–162°C.) applied in aqueous medium containing 0.1 per cent. w/v sulphonated loral and 10 per cent. v/v acetone. The average deposit on the dish was 7 mg./sq. cm. of aqueous medium containing different concentrations of rotenone.

Samples of adult, apterous, parthenogenetic females from each type of host-plant were sprayed with a series of concentrations of pure rotenone in the laboratory with a Potter tower (Potter, 1952). The insects were removed from the host-plants and placed in a 9-cm. petri dish containing a substratum of tricolene cloth and sprayed. They were then confined by muslin covers in the sprayed dishes until examination. Ten separate comparisons were made and in the last three tests the insects were fed with broad-bean leaves after the spray deposit had dried.

The dishes were kept at 20°C. and 50–60 per cent. R.H. for 24 hr. (48 hr. 8.vii.53) and then inspected on a constant temperature warm plate (Tattersfield & Potter, 1943). Three replicates, each containing approximately 15 Aphids were used at each concentration. The relative sizes and general condition of the two stocks, from broad bean and clover, were generally noted but no measurements were made. On most occasions the Aphids from clover appeared smaller and less active than those from broad bean. This point of difference in size is dealt with later on.

Results.

The results of the ten separate comparisons referred to above, subjected to probit analysis, are set out in Table I and show that the ratios of resistance of the Aphids from clover to that of the Aphids from broad bean varied from 0.9 to 2.5, estimated at the concentrations required to give a 50 per cent. mortality of individuals under the conditions of the tests. In five tests the Aphids reared on clover were significantly more resistant than those reared on broad bean. In the remaining five tests, four also indicated that the clover-reared Aphids might be more resistant than those from broad bean, but the difference was not significant. In the test on 7.vii.52 the Aphids from beans appeared slightly more resistant than those from clover, but there was practically no difference between the two.

Proportion of Dry Matter in Aphids reared on Clover and Broad Bean, respectively.

It was thought that, although the Aphids from broad bean were usually larger than those from clover, it was possible that the difference in size was due to a higher liquid content and that if the comparison were based on dry matter the difference might not be so great. It seems also that if the proportion of dry matter differed with the host-plant on which the insect was reared, this would provide evidence that nutritional differences were being produced which could affect resistance.

An estimate was made of the dry weight as a percentage of the wet weight of Aphids reared on broad bean and on clover. Batches of approximately 20 Aphids were weighed and then dried in an oven at 67°C. and reweighed. Drying was continued until the change in weight between successive weighings was very small, or there was no change. The results obtained are given in Table II. It appears that the Aphids from clover have a small but significantly higher percentage of dry matter. A comparison of toxicity based on dry matter would differ only slightly from that based on wet weight. But it is still possible that the increased percentage dry weight of the Aphids from clover may be important, since the figures provide evidence that the different host-plants are producing

differences in the composition of the Aphids that feed on them. It is reasonable to assume that the differences in composition, which could only be caused by differences in nutrition, could lead to differences in resistance.

TABLE II.

Difference in percentage dry weight of *A. pisum* reared on broad bean and on clover.
Insects oven-dried at 67°C.

Test no.	Period of drying (min.)	Broad bean			Clover		
		Wet weight	Dry weight	Dry weight as % of wet weight	Wet weight	Dry weight	Dry weight as % of wet weight
1	170	0.05395 0.05190	0.01145 0.01140	21.22 21.97	0.03585	0.00900	25.10
2	105	0.05675 0.06605	0.01335 0.01555	23.52 23.54	0.03055	0.00800	26.19
3	285	0.05890 0.05705	0.01240 0.01190	21.05 20.86	0.03337 0.03180	0.00760 0.00760	22.77 23.90
4	210	0.06100 0.05580	0.01415 0.01160	23.20 20.79	0.03415 0.03630	0.00890 0.00930	26.06 25.62

Mean difference in percentage dry weight/wet weight (clover-broad bean) is 3.1 ± 0.34 which is significant at 1% level.

Approximately 20 Aphids per weighing bottle.

Discussion.

Since the Aphids from clover were generally smaller than those from broad bean, the amount of spray retained will be different and this may affect the percentage kill. Some measurements were made of the amount of spray retained by insects of different sizes and these are set out in Table III.

The regression of the weight of poison retained on the weight of the individual was calculated. The equation of the line is $y = 0.084 + 0.137 x$, where y is the weight of poison retained, and x is the weight of the individual. The standard error of b , the slope, is 0.014 (d.f. 10). The slope given by the line is significant at a 0.1 per cent. level, and shows that the weight of deposit increases as the weight of the individual increases, within the limits tested.

The regression of the weight of deposit of poison per unit weight of insect on the weight of the insect was also calculated. The equation of the line is $y = 0.358 - 0.035 x$, where y is the weight of deposit per unit weight of insect, and x is the weight of the individual. The standard error of b , the slope, is 0.011 (d.f. 10). The slope given by the line is significant at a 2 per cent. level, and shows that the weight of deposit per unit weight of insect decreased as the total weight, and hence the size of the individual, increased.

It therefore appears that with large individuals the amount of poison retained per individual will be larger than with small individuals, but that the amount of poison per unit weight of an individual will be larger with smaller individuals than with larger individuals. The regression lines were calculated from the 12 sets of data shown in Table III.

From the foregoing data it appears that the insects from clover are more resistant than those from broad bean when judged on the number of individuals killed by a spray applied evenly over a surface on which the Aphids are placed.

With this procedure, the Aphids from clover, being smaller, receive a greater weight of poison per unit weight of insect than those from broad bean, although the larger individuals from beans receive a larger total dose. The difference in resistance might therefore be greater if the poison were applied so that each individual had an equal weight of poison per unit weight of insect.

TABLE III.

Weight of spray retained by Aphids of different sizes.

No. of Aphids in sample	Mean weight of Aphid (mg.)	Mean weight of deposit on Aphid (mg.)
10	2.72	0.43
10	2.47	0.41
10	2.49	0.48
10	1.55	0.26
10	1.57	0.31
10	1.53	0.26
10	1.70	0.35
20	1.10	0.25
20	0.99	0.20
20	1.21	0.23
20	0.87	0.23
20	0.93	0.22

The obvious reason for differences found in resistance is that the larger individuals from broad bean are retaining a greater total weight of poison than the smaller individuals from clover. The notes in Table I, however, do not indicate that the difference in resistance becomes larger as the difference in size between insects reared on broad bean and clover, respectively, becomes greater but rather that it occurs irrespective of any size differences. If the cause is not primarily associated with size differences, the assumption might be made that it is due to nutrition. The figures given in Table II, which show that the clover-reared Aphids have a greater proportion of dry matter in their composition than those reared on broad bean, indicate that differences due to nutrition are being produced. These may account for the difference in resistance.

Summary.

By means of a laboratory spraying technique, ten comparisons were made of the resistance to rotenone of samples of adult apterous viviparous parthenogenetic females of the Pea Aphid, *Acyrtosiphon pisum* (Harris), reared on broad bean and on clover, respectively. In nine of the tests, the Aphids from clover were more resistant than those from broad bean, the ratios ranging from 1.1 to 2.5. These differences were not all significant. In the remaining test the Aphids from broad bean showed a very small increase in resistance over the Aphids from clover which was not significant.

The Aphids from the clover were generally smaller than those from broad bean. Figures are given to show that while the total amount of poison retained by the larger individuals from broad bean was more than that retained by the smaller individuals from clover, the amount of poison retained per unit body weight was greater with the smaller individuals. It appears, therefore, that while the results obtained might be due, at least partially, to the greater total weight of poison retained by the larger individuals from broad bean, the difference in resistance between the insects from broad bean and clover, respectively, might be even greater if the poison were applied on the basis of equal weight of poison

per unit of body weight. Since the difference in resistance between the Aphids from the two host-plants did not appear to depend primarily on difference in size, the assumption might be made that it is due to difference in nutrition. It was found that Aphids reared on clover had a significantly higher proportion of dry matter in their composition than those reared on broad bean, which may be taken as evidence that differences due to nutrition are being produced. These may lead to differences in resistance.

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THE EFFECT OF OBSTRUCTIVE CLEARING ON *GLOSSINA PALPALIS* (R.-D.).

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Introduction.

In the savannah belt of West Africa, the distribution of *Glossina palpalis* (R.-D.) is confined to the vicinity of the riverine vegetation. Over a period of six years, a population of this tsetse was studied in great detail along 2,400 yards of stream, situated 15 miles north of Kaduna, in Northern Nigeria. The results from this ecological study have been published (Nash & Page, 1953), and amongst them were observations on the popularity of different types of riverine vegetation and an assessment of the requirements of *G. palpalis*. It was concluded that this tsetse needs good vertical insulation from the sun as provided by a high canopy, good lateral insulation from sun and wind as provided by creeper-curtain or steep stream banks, and a free flight-line along the stream-bed so that in the hot dry season it can fly from crossing-place to crossing-place in search of food. It was suggested that if the stream-bed was choked by felling the trees forming the canopy so that they fell into it, the destruction of both vertical insulation and free flight-line might together succeed in making the habitat untenable in the dry season, the tsetse being forced to seek for food in the adjacent savannah at a time of year when the climate had been shown to be lethal. The idea was to spare the giant emergents and all thicket species and creepers so that the latter could rapidly cover the fallen trash and produce a tangle that would be impenetrable to the fly.

The existing method of riverine clearing employed in Nigeria is that known as partial clearing. Only the undergrowth and low-branching trees are removed, permitting the entry of the hot, dry harmattan wind and the dissipation of the essential riverine ecoclimate (Nash, 1940). The method has been used with complete success since it was first introduced in 1937, but it suffers from one big disadvantage. It is essential that regeneration be checked by piling and burning the trash over the cut stumps, which has now become an exceedingly costly process, and also by regular re-slashing to keep down the vegetation which survived the initial burn.

If the new obstructive clearing method were to succeed in eradicating *G. palpalis*, it would reduce the initial cost of clearing by removing the piling and burning stage, and obviate the need for re-slashing because it should be many years before the vegetation returned to its normal structure—by which time a vast area of country could have been rendered fly-free, and further suppression of the vegetation would be unnecessary. It was appreciated, however, that this new method was unlikely to succeed against *G. tachinoides* Westw., a thicket species which can survive in the absence of high shade; nevertheless, there are vast areas of West Africa from which this species is absent.

A grant from the Colonial Development and Welfare Research Funds, which

was to be spread over three years, made it possible to undertake a pilot scheme to elucidate the effect of obstructive clearing on *G. palpalis*; the results of this experiment form the basis of the present paper.

The River Kuyi system, south of Kaduna, was selected in an area some 20 miles south of Katabu where the original research has been carried out. Clearing was undertaken in two dry seasons, and as the plan of attack employed in the two years was different, it is best to describe the results as if they were two separate experiments.

The River Matari was used as the control stream, and although belonging to a different system it is situated only a few miles from the experimental area.

The First Year's Experiment.

Description of the experiment.

Two thousand three hundred yards of the R. Kuyi and 1,100 yards of its tributary—the R. Tawawu—were selected for the first year's experiment. For fly-round purposes the streams were divided into 100-yard-long sections, the limits of which, for convenience, were marked on the ground by trees numbered from 0 upwards, so that the first section was between Trees 0-1 (see fig. 1). This

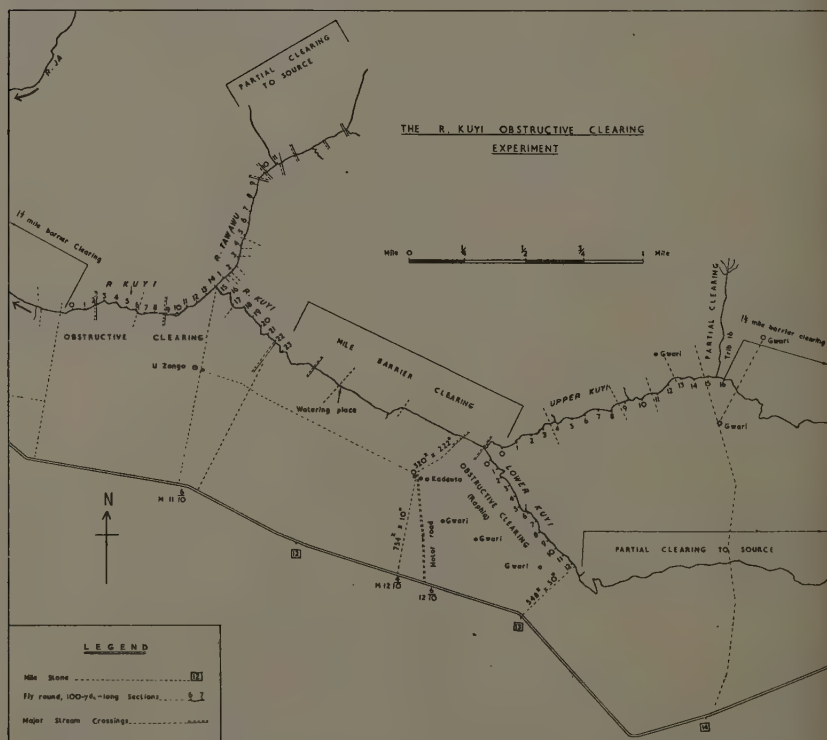


Fig. 1.—The River Kuyi system, showing the stretches to which experimental obstructive clearing was applied and the barrier clearings that isolated them. Apart from named villages, groups of huts of the local Gwari tribe are shown (Gwari).

stretch of stream was chosen because it afforded a fairly uniform and continuous canopy, beneath which was an undergrowth rich in creeper and thicket species which might be expected to smother the trash after the canopy had been felled. Notes on the vegetation are given in Appendix I, but as it was impossible to enlist the help of an expert botanist, all the species present could not be identified. It should be noted that the common raphia palm was virtually absent.

Sections between Trees 0-23 on the R. Kuyi run mainly through farm land, but the lower reaches of the R. Tawawu run through *Isoberlinia doka* woodland.

The experimental reaches, which totalled almost two miles in length, were isolated as follows. The R. Tawawu was partially cleared to its source. Both upstream from Tree 23 and downstream from Tree 0 the experimental reach of the R. Kuyi was isolated by means of mile-long ruthless barrier clearings—the distance being a straight distance and not measured round the bends. As will be described later, the lower barrier had to be extended to $1\frac{1}{2}$ miles in the following year.

The purpose of this first experiment was to ascertain whether *G. palpalis* could survive in the new environment, and not to complicate the issue by introducing other factors, even though such factors would be normal practice in schemes of tsetse eradication. Thus, instead of clearing the three barriers *outwards* from the experimental reach and so driving their fly populations away, we cleared them *inwards* and drove the tsetse population of five miles of stream (measured round the bends) into the two miles forming the experimental reach.

The effect of obstructive clearing upon the tsetse.

Effect on fly populations.—The results are shown graphically in fig. 2. The rainfall figures given were recorded at Kaduna which is eight miles away. A comparison between the fly density of the control stream, the R. Matari, and that of the R. Kuyi and R. Tawawu shows that before clearing started the fly populations of both the experimental streams fluctuated normally. High fly densities were recorded in September 1953 (the late rains), followed by the usual drop at the end of the rains which continued with the advent of the dry season in November. (This is exactly as shown by Nash & Page, 1953, p. 136, fig. 14.)

As was expected, by clearing the barriers inwards, the fly population was artificially increased in both the experimental streams, at a time when fly density was decreasing on the control as a result of the rigours of the dry season (see December and January). The periods covered by the construction of both barriers and obstructive clearings is shown on the figure by hatched columns.

On both streams, the immediate effect of obstructive clearing was to produce a great drop in fly density, but not rapid extermination as had been anticipated. It was expected that the tsetse would be immediately forced out into the woodland in search of food and would succumb to the high temperature and very high evaporation rate; however, on 21st January 1954, three herds of nomadic Fulani cattle arrived, settled near the stream junction, and remained throughout the whole dry season and early rains until 1st May, by which time the outside climate was no longer lethal. Daily, these cattle would graze on the new grass along the experimental reaches, offering an assured food supply to the tsetse lurking under the trash. The streams never dried up, and meteorological readings showed that climatic conditions under the trash were well within the range tolerated by this tsetse fly, even in the late dry season.

It will be noted that in May and June 1954 the usual, rapid, wet-season increase in the fly population took place on the control, but not on the two experimental streams; clearly the alteration in the vegetation had made this impossible.

By August, eradication had been achieved on the R. Tawawu, but not on the

R. Kuyi. It was noted that the flies which persisted on the Kuyi tended to be concentrated at the two ends where they abut on the barrier clearings. (Out of the 34 one-hundred-yard-long sections into which the experimental reaches are divided, three-quarters of the fly population were found in the five terminal sections adjoining the beginning of the Kuyi barriers.) This strongly suggested an influx of flies from across the barriers in the heavy rains, a supposition which was proved by marking experiments.

Between mid-July and mid-November, 729 flies were marked and released in the *uncleared* stream above and below the upstream and downstream barriers, respectively. Four of these marked flies crossed the barriers between mid-July and mid-September. During this period many more flies must have crossed the

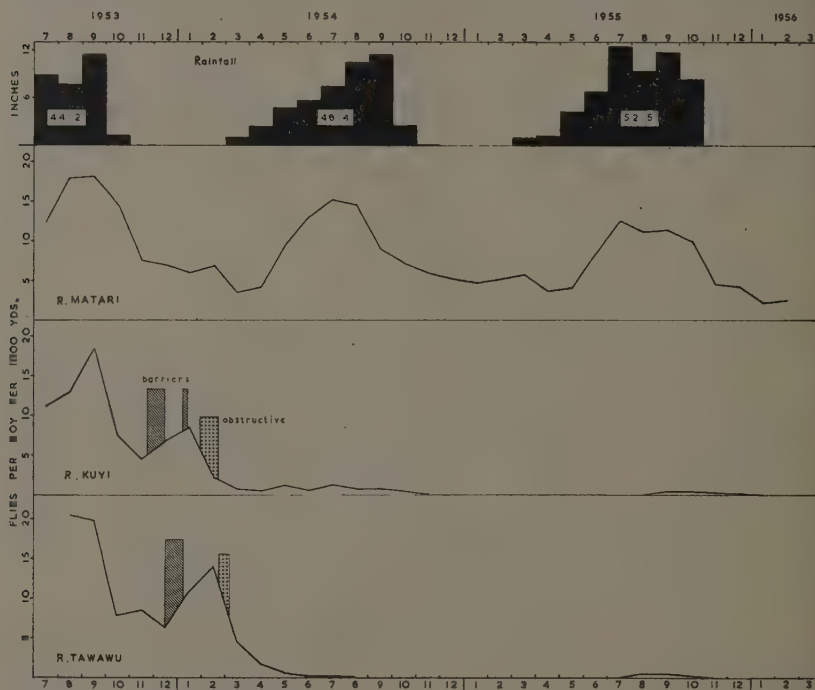


Fig. 2.—The effect of obstructive clearing upon the population of *G. palpalis* on the R. Kuyi and R. Tawawu. The R. Matari (control) shows the normal seasonal fluctuations in fly density.

barriers, but being unmarked would have been unrecognisable as immigrants. Marking in the marking grounds took place at fortnightly intervals, the same interval as that elapsing between pairs of fly-round catches on the experimental stream. Since even in the marking areas the proportion of marked flies recaptured to unmarked flies was as 1 is to 10, it is fair to estimate that the 4 marked flies which were known to have crossed the barriers represented at least 40 flies that had in fact got across. Only 64 flies were caught in the experimental area during the whole of the marking period; of these at least 40 are believed to have been immigrants, and the balance of 24 may well have been progeny of the earlier

immigrants. The results suggest that had the length of the barriers been adequate, extermination might have been achieved on the Kuyi about August, as it was on the Tawawu.

With the start of the dry season in November 1954, the crossing by *G. palpalis* of treeless barriers became impossible; it is significant that in the following month not a fly was caught on the Kuyi. Unfortunately, three flies were caught on 19th February 1955, but these were undoubtedly carried by a labour gang which on that same day had been sent down from the fly-infested Upper and Lower Kuyi to re-slash the previous year's barriers. Any other flies introduced at the same time must have died rapidly, as no more were caught for the next seven months.

In the meanwhile, the upstream Kuyi barriers had virtually been extended because the Upper and Lower Kuyi had been obstructively cleared as part of the second year's experiment. To the west, the downstream ruthless barrier was extended for a further half mile, making a total of $1\frac{1}{2}$ miles; from this point the Sleeping Sickness Service continued the clearing down to the Kaduna river, as part of their programme for this area. At the very end of our barrier, a tributary—the R. Ja—was discovered, and believing that it flowed from due north it was cleared for only a quarter of a mile, which should have put $1\frac{1}{2}$ miles of ruthless barrier clearing between the nearest fly and the beginning of the experimental section of the Kuyi.

In August 1955, again the height of the rains, tsetse suddenly re-appeared in the Tawawu which had been fly-free for a complete year. Investigation revealed that, further upstream, the R. Ja, instead of coming down from the north as supposed, came from due east, having passed within one mile of the R. Tawawu. It is believed that flies, flooded out of the R. Ja by the exceptional rainfall of July 1955, were driven into the woodland and across to the Tawawu. (That such a feat is possible has previously been shown by Nash & Page, *loc. cit.*)

In September, the Kuyi became re-infested, and confirmatory evidence that the R. Ja was responsible was obtained by the recapture of a fly on the Kuyi that had been marked and liberated on the Ja.

By January of the following dry season (1956), the R. Kuyi was once again fly-free and has remained so to date (31st March 1956). It is interesting to note that despite wet-season reinvasion, the conditions two years after clearing are still such that fly is exterminated in the dry season.

It is a pity that once again in the history of experiments on tsetse reclamation the picture has been marred by a failure to obtain complete isolation at all seasons, but the obtaining of such conditions eats heavily into any grant for a pilot scheme. It is hoped, however, that the fair-minded reader will admit that, from the evidence produced, this first year's experiment gave every indication that, providing isolation is complete, obstructive clearing does result in eradication, as witness the Tawawu being fly-free for 12 months.

TABLE I.

The percentage of the total population that were hungry.

Stream	Month before clearing	Month after clearing
Matari (control)	49 (73 flies)	38 (42 flies)
Kuyi	36 (158 ")	55 (18 ")
Tawawu	32 (47 ")	65 (20 ")

Effect on nutrition.—The effect of obstructive clearing upon the nutrition of the fly was also studied. Owing to the small numbers of flies which survived the clearing, it has been impossible to follow the normal practice of basing hunger-staging on old males only; instead, the figure used has been the percentage of the total population staged as "hungry".

The immediate effect of the clearing can be seen in Table I.

Hunger decreased during this period on the control stream, but there was a marked increase in the percentage of hungry flies on the two experimental streams, even though the cattle, referred to earlier, provided an abundant food supply. The state of hunger continued to be greater on the Kuyi than on the control until the height of the rains; but comparable observations on the Tawawu were rendered impossible, because eradication was achieved so quickly.

Effect on sex proportion.—The immediate effect of the obstructive clearing on the sex proportion is shown in Table II.

TABLE II.

The percentage of the total population that were females.

Stream				Month before clearing	Month after clearing
Matari (control)		49 (73 flies)	47 (42 flies)
Kuyi	48 (158 ")	50 (18 ")
Tawawu	21 (47 ")	60 (20 ")

There was an immediate and striking increase in the percentage of females on the Tawawu but not on the Kuyi; however, if the comparison is taken over a longer period, we find that the percentage of females on the Kuyi became much greater after clearing: thus, in the five months before the clearing started, the percentage of females on the Kuyi averaged 34, but in the following five months the average was 53 (based on 87 flies); the comparable figures for the control were 39 and 40, respectively.

The effect of obstructive clearing upon the vegetation.

After the clearing had been completed, the felled material appeared as a long mound of leafless branches arching from one bank across to the other, and interspersed with living but damaged shrubs that had never been cut down. The flight-line was blocked; there was no continuous overhead canopy, but only the shade from the occasional tall emergent that had been spared. Within a few weeks, creepers appeared among the trash, *Mucuna pruriens* being much in evidence. By July, four months after clearing, the trash was completely smothered in creepers and the stream-bed quite impenetrable, except for the villagers' fords and watering places which had been left unobstructed.

Twenty two inches of rain fell in August and September 1954. During this period the experimental streams quite frequently overflowed their banks, as they had always done, but the flooding only lasted for a few hours; no complaints were made by the local farmers, and there were no indications of damage having been done to the stream banks. The trash tended to sink down within the stream-bed, and much temporary damage was done to the new creeper growth; in a few places, the stream-bed became visible owing to the shifting of trash, but the force of the water never tossed the trash up on to the banks or formed dams

through which the water could not percolate. There is no doubt, however, that in very hilly country with a rapid run-off such an effect might be produced.

In the following dry season no attempt was made to control fire, with the result that small sections of the obstruction were burnt out: it is thought that, on perennial streams, the annual fires will make only small inroads.

By the middle of the next rains—July 1955—the general appearance was still that of a blocked stream-bed, occasionally interrupted by gaps, 10–20 yards in length, in which the stream-bed had become exposed. However, from the tsetse's point of view there was nothing in the slightest comparable to the original free flight-line beneath a heavy overhead canopy, along which the insect could search for food. The insect would now have to fly, fully exposed to the sun, along a few yards of stream-bed and then over the top or along the sides of long distances of obstruction, before again finding an open path over water. Whereas such a method of searching for food would present *G. palpalis* with no difficulty under the overcast conditions of the heavy rains, it would be impossible in the hot dry season.

By the dry season of 1956 it was apparent that many more small gaps, 5–20 yards in length, had appeared in the stream-bed. Larger gaps only occurred in the first 1,400 yards, which was obstructively cleared before we had appreciated the necessity of leaving whole trees in the stream and not cutting up the trash into small lengths. Nevertheless, in a stream as large as the R. Kuyi, it can only be a matter of time before the obstructions are eventually removed by flood water. However, its tributary, the R. Tawawu, is still extremely well obstructed two years after the original clearing was made.

The present trends suggest that the vegetation of small tributary streams may well be converted into impenetrable thicket which may persist for many years, but that on larger streams with a considerable flow of water the obstructions will slowly disintegrate and that the final appearance may be that of a partially cleared stream which has not been re-slashed.

The effect of obstructive clearing upon the natural hosts.

Records have always been kept of the game and spoor seen upon the fly-rounds. Figures, when quoted, will cover the six months before clearing—July to December inclusive—and the same six months after the clearing had been completed.

TABLE III.

Records of wild animals, July–December, in two years.

	R. Kuyi		R. Tawawu	
	Before	After clearing	Before	After clearing
Occasions on which duiker spoor seen	25	6	21	9
Occasions on which monitor lizards seen	10	23	2	0

The general results indicate that cattle are frequently seen along both streams between December and April; in May the Fulani cattle owners move northwards with their herds. Man is a common species throughout the year, but much more so on the Kuyi than on the Tawawu. The incidence of man is not likely to be affected by obstructive clearing, except in so far as man may cease to visit the uninhabited reaches in search of forest produce such as bamboos; the records bear this out in that on the Kuyi, which supports a village, there was no decrease

in the number of observations of men and tracks seen, but there was on the R. Tawawu. (R. Kuyi: before = 82, after = 80; R. Tawawu: before = 22, after = 7.)

Of the species of wild animals, the only ones that occur as regular hosts in this fairly well populated area are duiker (*Sylvicapra grimmia coronata* and *Cephalophus rufilatus*) and the monitor lizard (*Varanus niloticus niloticus* and *V. exanthematicus*); for duiker, records of spoor are more reliable as these small antelopes hide in the stream-bed during the day and are rarely put up, whilst for the *Varanus* one must rely on the number of lizards seen as they rarely leave visible spoor. The results are shown in Table III.

It would appear that the effect of obstructive clearing was to reduce the duiker population considerably, an impression that will be confirmed when reviewing the results for the streams cleared in the second year. On the other hand, monitor lizards, which were only common on the Kuyi, were seen more often after the clearing.

The Second Year's Experiment.

Description of the experiment.

In the first year's experiment, ideal vegetational conditions were selected in that there was a fairly continuous overhead canopy which, after felling, would choke the stream-bed, and also an abundance of thicket and creeper species which would later smother the trash.

As part of the second year's experiment, 1,200 yards of the Lower Kuyi (see fig. 1) were chosen because of the great variation in the density of the vegetation and because conditions were far from ideal. Along three-quarters of the length, the riverine vegetation was too scanty to provide material for good, continuous obstruction of the stream-bed; but along the remaining quarter it was excessively thick and heavy, frequently extending up to 50 ft. from each bank. After removal of the canopy, these latter conditions might be expected to regenerate into a shrubbery, suitable for *G. palpalis*. Further, the palm, *Raphia sudanica*, was the dominant species, and, owing to its very rapid rate of regeneration, the restoration of an overhead canopy might be expected to take place very quickly. The Lower Kuyi runs through woodland in a steep-sided valley, and tends to be marshy.

In addition, 1,600 yards of the Upper Kuyi were chosen for obstructive clearing, because its vegetation was not very different from that dealt with in the first season; hence in a sense it would act as a control. The last 400 yards only of this stream run through farm land.

The experimental reaches, which totalled $1\frac{3}{4}$ miles, were isolated at their upstream ends in the following manner. The Lower Kuyi was partially cleared up to its source, and the Upper Kuyi was isolated by means of a $1\frac{1}{2}$ -mile long ruthless barrier clearing.

Having shown in the first year's experiment that the conditions produced by obstructive clearing were almost certainly intolerable for *G. palpalis*, the purpose of the second year's work was to ascertain how quickly the fly could be eradicated when following normal reclamation practice, i.e., the obstructive clearing being undertaken first and then the barriers cleared *outwards* from the experimental reach, thus driving their populations away from the experimental streams, instead of into them as was done in the first year.

The effect of obstructive clearing upon the tsetse.

Effect on fly populations.—The results are shown graphically in fig. 3. Within less than three months, eradication had been achieved on both streams; during this period only 19 flies were caught. The two streams have now been

fly-free for a year. The excellence of these results is attributed to the fact that complete isolation was achieved.

Effect on nutrition.—Turning now to the effect of obstructive clearing upon the nutrition of *G. palpalis*, owing to the small number of flies caught after clearing it has been necessary to combine the totals for both Upper and Lower Kuyi for the three months which elapsed before eradication was achieved

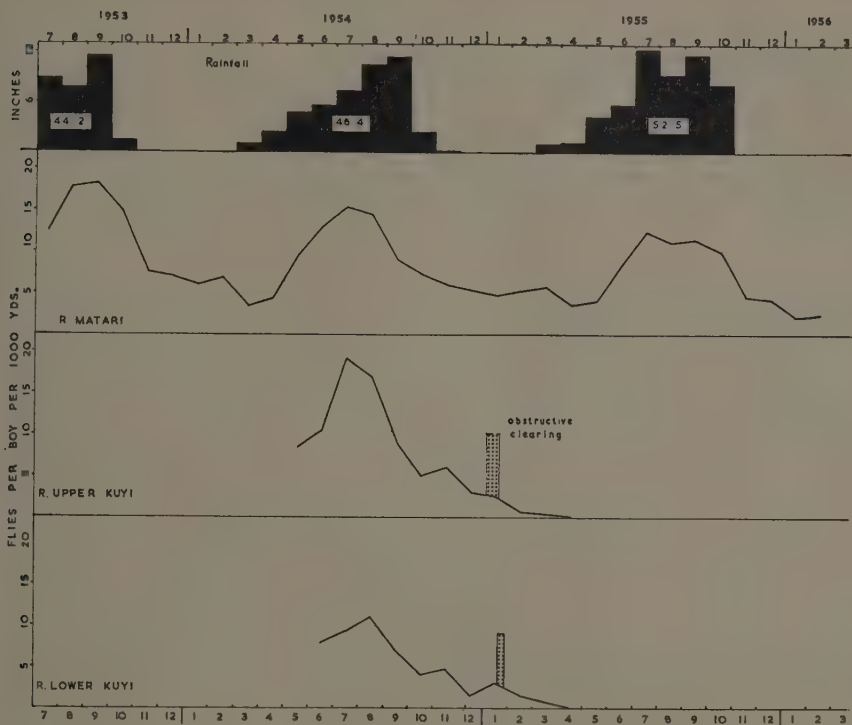


Fig. 3.—The effect of obstructive clearing upon the population of *G. palpalis* on the R. Upper Kuyi and R. Lower Kuyi. The R. Matari (control) shows the normal seasonal fluctuations in fly density.

Comparing the state of nutrition in the three-month period before clearing with that after clearing, it would seem that hunger increased enormously as a result of the clearing, but on the control the percentage did not vary. It should be recorded that cattle were far fewer on these streams than they had been on the streams cleared in the previous year.

Effect on sex proportion.—The effect of obstructive clearing on the sex proportion is shown below in Table V.

Since the percentage of females increased considerably on the control, it would be unwise to draw any conclusions from these figures.

If the data on nutrition for both years' clearings are considered together, there would appear to be little doubt that the obstructive clearing of the habitat leads to a great increase in the hunger of the surviving flies; there may also be a tendency for the percentage of females to increase, but this is less certain.

The effect of obstructive clearing upon the vegetation.

As had been expected, by the end of the first rains the appearance of the obstructive clearing along the Lower Kuyi was less satisfactory than that observed on the streams dealt with in the first season's work. The initial scarcity of creeper and thicket species along this stream was reflected in a greatly reduced creeper-smother of the trash; however, the trash, although sunken into the stream-bed, had not shifted. The raphia palms had regenerated rapidly, varying

TABLE IV.

The percentage of the total population that were hungry.

Stream	Three months before clearing	Three months after clearing
Matari (control)	47 (223 flies)	47 (178 flies)
Lower and Upper Kuyi ..	41 (144 flies)	79 (19 flies)

in height from 5 to 16 ft. and averaging about 10 ft.; clearly where this species is dominant the restoration of a high canopy will be much quicker than elsewhere. In contrast, the Upper Kuyi presented an almost perfect picture of obstructive clearing; none of the trash had shifted, there was an excellent creeper growth and considerable regeneration from stumps, especially from those of *Berlinia heudelotiana*, *Uapaca somon* and *Adina microcephala*. *Mucuna pruriens* was the dominant creeper species.

TABLE V.

The percentage of the total population that were females.

Stream	Three months before clearing	Three months after clearing
Matari (control)	34 (223 flies)	52 (178 flies)
Lower and Upper Kuyi ..	31 (144 flies)	63 (19 flies)

The effect of obstructive clearing upon the natural hosts.

Both man and cattle were recorded less frequently on the Lower Kuyi than on the Upper Kuyi; however, cattle were present only seasonally.

On both streams the number of observations of men and tracks seen was considerably less during the six months—July to December—after clearing, than it was for the same six months before, presumably because of the destruction of the forest produce. (Lower Kuyi: before = 12, after = 5; Upper Kuyi: before = 20, after = 8.)

In the six months (July–December) before clearing, duiker spoor was recorded 26 times on the Lower Kuyi but only 7 times in the same six months after clearing; comparable figures for the Upper Kuyi were 18 and 0. Here again, obstructive clearing would seem to have reduced greatly the duiker population, the effect being most noticeable on the streams where man was most commonly encountered. It is probable that the obstruction of the tsetse's flight-line also results in the blockage of the duiker's escape route along the stream-bed, and so renders the duiker more vulnerable to man and his dogs.

Monitor lizards were scarce on both streams before clearing with only five

records on each, and even scarcer after clearing when the records dropped to 1 and 2.

The results from both years' experiments suggest that obstructive clearing leads to a reduction in the human hosts available along the stream banks, except in the vicinity of a village; further, that it leads to a considerable reduction in the duiker population, which is most marked near human settlement.

The Technique used for Obstructive Clearing.

Before any clearing is started, the grass should be well burnt along both banks, because the trash in the stream-bed will be highly inflammable until the rains have broken.

The gang should consist of, say, 40 labourers with one intelligent headman and two sub-headmen. It should be sub-divided into two parties consisting of 24 "axemen" and 16 "trimmers".

The clearing operations should be divided into three stages. In Stage I, axemen and trimmers work together and cut out the small saplings, thus improving the visibility. In Stage II, the axemen fell the trees of the canopy. In Stage III, the trimmers clear up after the axemen.

Stage I.

On the first day the whole gang works together, half on one bank and half on the other; the headmen allocate small trees, up to 4 inches in diameter, which the labourers fell. Creepers and small thickets are spared. Forty men can do about 2,000 yards per day of Stage I clearing on streams supporting a medium fringe of vegetation.

On the second day of operations, only the trimmers do Stage I clearing, the axemen starting on Stage II work. On the third day the trimmers will start on Stage III, which is their real work.

Stage II.

This is the skilled part of the technique, and consists in felling all the trees that form the canopy so that whenever possible they fall into the stream-bed. Giant emergents which protrude above the canopy, and any medium-sized trees which have practically no crown, are spared.

There is no point in roping trees which lean well out into the savannah, nor those which are obviously going to fall into the stream-bed, but those whose line of fall is in doubt are roped from a point high up in the crown. It is the headman's duty to see that a rope is manned by the other axemen shortly before a tree is about to fall.

Steering a tree so that it will fall into the stream-bed depends upon laying the rope at the correct angle; this angle is invariably some 25° more than one's instinct tells one. Quite wrongly one visualises that the falling tree will pivot on its stump in answer to the pull, but as soon as the tree starts to fall the rope slackens and no more influence can be exerted.

Trees that are 18 inches or more in diameter should not be roped, as little influence can be exerted on their line of fall.

Twenty four axemen are far too many to have working in the same spot if the headman is to prevent trees falling before the ropes are manned. It is therefore necessary to detach some of the party under the sub-headmen and send them on ahead to fell all medium-sized trees which will obviously fall into the savannah, and also those trees which will obviously fall into the stream. The completion of such preliminary work makes it much easier for the headman's party to deal with the roped trees. The order in which such trees should be cut needs intelligence on the part of the headman if he is to avoid having a large, partly-fallen tree supported on a smaller tree which no one will be keen on felling.

The stems of lianas and creepers are only cut when they prevent a tree from falling. Small thickets and shrubs are spared; they get badly crushed but soon recover.

Stage III.

The purpose of the 16 trimmers is to clear up after the Stage II gang. Their first job is to tumble in those trees which just failed to fall into the stream; this can often be done by tying a rope to the crown and pulling from the opposite bank, or in the case of small trees by lifting, rolling and pushing.

It is most important to try to get as many whole trees as possible into the stream-bed and to tumble in large limbs cut from the crowns of trees that fall well outside; the limbs should not be cut up into small pieces. The foundation will thus be solid and rendered immovable by flood water which can readily percolate through; but if the stream is choked with small branches these will be washed down to form dams, leaving long unobstructed gaps.

The final appearance of an obstructively-cleared stream should be a mound of great branches arching from one bank across to the other, providing a solid framework to support the regenerating creepers, which will soon smother the trash and block the tsetse's flight-line (fig. 4).



Fig. 4.—The final appearance of an obstructively-cleared stream.

The Relative Cost of Obstructive Clearing.

The following costings are for both seasons combined. The cost of 8.4 miles of ruthless and partial barrier clearings worked out at 678 man days per mile. This compares closely with the figure of 664 man days per mile, obtained for similar types of clearing carried out along 540 miles of stream in the Anchau Corridor (Nash, 1948). The similarity of the figures suggests that the density of the riverine vegetation dealt with in these experiments was typical of that found in the north of Northern Nigeria.

The cost of obstructive clearing along 3.75 miles of stream worked out at only 390 man days per mile, which is only 58 per cent. of the cost of the traditional methods.

Our figure of 390 man days per mile would have been even lower had it not been for the three following circumstances. In the initial stages the headmen were having to be taught and we were having to work out the best technique. The labourers belonged to a non-Hausa-speaking tribe, so that instruction had to be carried out mainly in dumb-show. The labour gang had to be completely changed every fortnight, when teaching had to start all over again.

The big saving in the new method is that the tidying-up work of the trimmers is far less expensive than the usual construction of bonfires over the stumps, or the digging out of the stumps, both being measures designed to reduce regeneration.

Discussion.

The experiments described above are not completed; the interval between the initial clearing and the time when the vegetation again becomes favourable to *G. palpalis* may not be known for some years.

The old technique of partial clearing combined with ruthless barriers is undoubtedly the most permanent method, but suffers from the grave disadvantage of high initial cost and the necessity for years of subsequent maintenance. This is still, however, the only method that can be safely used for the protection of individual villages. In such small-scale schemes, obstructive clearing would be useless because in the height of the rains flies would invade the clearing from the uncleared stream.

When dealing with large-scale projects, if it is decided that the old clearing methods have become too expensive and their maintenance too onerous, one is left with the alternatives of attack by insecticides or by obstructive clearing.

In the case of insecticides, if complete isolation is not achieved at the first attempt, the stream system will have to be re-sprayed; but if obstructive clearing is employed, any wet-season immigrants will be destroyed in the following dry season, giving one several years at least in which to seal off the area and achieve complete isolation.

Whether the disintegration of small stretches of obstruction is of any importance to the fly is doubtful. The tsetse will still be unable to fly uninterruptedly in the ecoclimate along the stream-bed in search of food, and will still be without an overhead canopy.

It may well be asked whether in our experiments the results achieved could not have been obtained by the destruction of the overhead canopy, without resource to choking up the stream-bed. The answer is not known, but it is felt that, at any rate in the case of permanently flowing streams, *G. palpalis* would have survived the dry season in small numbers by sheltering under shrubs near water during the heat of the day and roving up and down the stream-bed in the early mornings and late afternoons in search of food. It seems reasonable to suppose that by denying the tsetse free passage within the ecoclimate, and by forcing it out into the exceedingly severe conditions experienced outside, one must have greatly increased its discomfiture.

Summary.

An experiment has been undertaken in Northern Nigeria to ascertain whether the felling of the trees forming the overhead canopy and the deliberate blocking, with trash, of the stream-bed to obstruct the tsetse's flight-line, would result in the eradication of *Glossina palpalis* (R.-D.).

Obstructive clearing was employed on approximately $3\frac{3}{4}$ miles of stream. The

results suggest that, provided the experimental reaches are adequately isolated, obstructive clearing does lead to the eradication of *G. palpalis*.

It is noteworthy that if, owing to inadequate isolation, the cleared stream becomes re-infested in the rains, conditions for at least the first two dry seasons are so unfavourable that flies cannot persist.

The immediate effect of obstructive clearing is to increase greatly the hunger of the few surviving flies.

Records suggest that obstructive clearing leads to a considerable reduction in the number of human hosts visiting uninhabited parts of the stream, presumably because of the destruction of forest produce. The duiker (*Sylvicapra* and *Cephalophus*) population also becomes greatly reduced, especially in the vicinity of hamlets. The hunger observed in the surviving fly population may therefore be in part due to an actual reduction in the number of hosts, as well as to the altered environment, which prevents free movement, under shade, of the hungry tsetse that is searching for food; instead, the tsetse is forced out into the open, where the climate in the dry season is intolerable, and presumably the unsuccessful fly rapidly succumbs from water-loss.

The effect of obstructive clearing on the vegetation is as follows. Within a few weeks the mound of trash is overgrown with creepers, *Mucuna pruriens* becoming dominant. In the first year's heavy rains the mound of trash tends to sink down in the stream-bed; on larger streams, spates do some temporary damage to the creeper growth and gaps may appear in the obstruction. The impression gained is that the blockage will persist for many years on small tributaries, but will disintegrate within a few years on larger streams. In very hilly country, with a rapid run off, the obstruction is likely to be displaced. In perennial streams of the type dealt with, the annual fires make only small inroads into the obstruction.

The technique evolved for the obstructive clearing of streams is described. The cost of this method worked out at 390 man days per mile, which is about half the cost of the present methods.

Should large-scale undertakings in the field confirm the efficacy of this new method, the biggest saving will be the elimination of the present necessity to re-slash streams that have been partially cleared. Observations will be continued to ascertain how long it will be before the vegetation again becomes suitable for *G. palpalis*.

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APPENDIX I.

Notes on the vegetation of the streams dealt with in the first year.

The River Kuyi.

Sections between Trees 0-14.

Trees The dominants were *Uapaca somon* and *Berlinia heudelotiana*. *Khaya senegalensis*, *Adina microcephala* and *Parinari Kerstingii* occurred occasionally.

Climbers	<i>Tetracera</i> sp. and <i>Landolphia</i> sp. were the dominant lianas. <i>Opilia celtidifolia</i> was quite common and <i>Sabicea</i> sp. occurred occasionally.
Small trees and shrubs of understorey	<i>Garcinia ovalifolia</i> was dominant and often reached 20 feet in height. <i>Olax</i> sp., <i>Santaloides gudjuanum</i> , <i>Carpolobia alba</i> , <i>Canthium venosum</i> and <i>Harungana madagascariensis</i> were not uncommon.
General	Tall, narrow-boled trees grew close together like huge saplings, producing a high canopy. A fair but variable creeper and shrub curtain existed near ground level, which aided the steep banks in providing dry-season insulation. The stream continued flowing throughout the dry season under observation, whilst in the previous rainy season it had been noted that the stream sometimes overflowed its banks, as indicated by débris.

Sections between Trees 14-23.

Trees	<i>Berlinia heudelotiana</i> and <i>Isoberlinia doka</i> were dominant, and <i>Syzygium guineense</i> common. <i>Adina microcephala</i> , <i>Khaya senegalensis</i> and <i>Harungana madagascariensis</i> also occurred. N.B. <i>Uapaca somon</i> was virtually absent.
Climbers	<i>Dalbergia</i> sp. was the dominant liana, <i>Strychnos</i> sp. was very common and <i>Landolphia</i> sp. also occurred. <i>Mucuna pruriens</i> and <i>Opilia celtidifolia</i> occurred occasionally.
Small trees and shrubs of understorey	The understorey tended to be far thicker than elsewhere. <i>Garcinia ovalifolia</i> was the dominant. <i>Olax</i> sp. occurred. <i>Carpolobia alba</i> was found in sections between Trees 16-17.
General	The vegetation of sections between Trees 14-23 was very different from that between Trees 0-14, as evidenced by the abundance of the liana <i>Dalbergia</i> sp. which was not found elsewhere, and the frequency of the liana <i>Strychnos</i> sp. Whereas the liana <i>Tetracera</i> sp., the <i>Uapaca</i> trees, and the shrub <i>Santaloides gudjuanum</i> were very common in sections between Trees 0-14, they were virtually absent in sections between Trees 14-23.

The River Tawawu.

Trees	The dominant trees were <i>Berlinia heudelotiana</i> , <i>Uapaca somon</i> and <i>Adina microcephala</i> . <i>Vitex doniana</i> , <i>Anthocleista nobilis</i> and <i>Khaya senegalensis</i> were common, and <i>Parinari Kerstingii</i> occurred.
Climbers	<i>Tetracera</i> sp. was the dominant liana, especially in the earlier sections. <i>Landolphia</i> sp., <i>Strychnos</i> sp. and <i>Opilia celtidifolia</i> were common, <i>Mucuna pruriens</i> was not uncommon whilst <i>Sabicea</i> sp. and <i>Smilax kraussiana</i> also occurred.

Small trees and shrubs of understorey	<i>Garcinia ovalifolia</i> was the dominant, reaching heights of up to 20 feet. <i>Phoenix reclinata</i> (never more than a few feet in height), <i>Canthium venosum</i> , <i>Carissa edulis</i> and <i>Harungana madagascariensis</i> also occurred. There were a few <i>Oxytenathera abyssinica</i> , but no <i>Raphia</i> sp.
General	Somewhat similar to sections between Trees 0-14 of the R. Kuyi, but the stream-bed being far shallower may account for the presence of the <i>Phoenix</i> palm, and for the fact that <i>Uapaca</i> is not quite so common. Certain species such as <i>Carpolobia</i> and <i>Santaloides</i> were virtually absent. In general, the riverine vegetation had less vertical curtain and less thicket. The R. Tawawu had much closer affinities with sections between Trees 0-14 of the R. Kuyi than with sections between Trees 14-23.

APPENDIX II.

Notes on the vegetation of the streams dealt with in the second year.

River Upper Kuyi.

Sections between Trees 0-16.

Trees	The dominants were <i>Berlinia heudelotiana</i> , <i>Syzygium guineense</i> , <i>Adina microcephala</i> and <i>Uapaca somon</i> . <i>Khaya senegalensis</i> and <i>Vitex doniana</i> also occurred, together with scattered <i>Ficus</i> sp.
Climbers	<i>Landolphia</i> sp., <i>Strychnos</i> sp., <i>Sabicea</i> sp. and <i>Opilia celtidifolia</i> were common, <i>Mucuna pruriens</i> and <i>Tetracera</i> sp. were also present.
Small trees and shrubs of understorey	<i>Oxytenathera abyssinica</i> occurred freely throughout. Common species were <i>Garcinia ovalifolia</i> , <i>Harungana madagascariensis</i> , <i>Canthium venosum</i> and <i>Carpolobia alba</i> . <i>Anthocleista</i> sp. and <i>Olax</i> sp. also occurred. There was practically no <i>Raphia</i> .
General	Very similar to the R. Tawawu except for the first five sections which were marked by a striking lack of undergrowth and shrubs, which tended to make "obstruction" difficult.

River Lower Kuyi.

This river was rather unusual as in a stretch of 1,200 yards the vegetation changed from extremely light growth to very heavy riverine forest up to 100 ft. wide. *Raphia sudanica* was dominant throughout.

Sections between Trees 0-8.

Trees	<i>Raphia sudanica</i> , <i>Berlinia heudelotiana</i> and <i>Uapaca somon</i> were common; <i>Syzygium guineense</i> , <i>Khaya</i> sp. and <i>Parinari Kerstingii</i> occurred.
Climbers	<i>Opilia celtidifolia</i> and <i>Mucuna pruriens</i> were dominant, <i>Landolphia</i> sp., <i>Tetracera</i> sp. and <i>Sabicea</i> sp. also occurred.

- Small trees and shrubs of understorey *Oxytenathera abyssinica*, *Garcinia ovalifolia*, *Anthocleista* sp. and *Carissa edulis* occurred frequently. *Carpolobia alba*, *Phoenix reclinata* and *Canthium venosum* were also present.
- General The riverine vegetation is extremely thin and may easily be seen through. There is no creeper curtain; the canopy is low, light and sometimes absent. This is not the sort of stream which would be deliberately chosen for obstructive clearing.

Sections between Trees 8-12.

- Trees *Raphia sudanica* and *Uapaca somon* were the dominants. Also occurring were *Syzygium guineense*, *Berlinia heudelotiana*, *Parinari Kerstingii*, *Khaya senegalensis* and *Vitex doniana*.
- Climbers *Tetracera* sp., *Strychnos* sp. and *Sabicea* sp.
- Small trees and shrubs of understorey *Oxytenathera abyssinica* was common. *Garcinia ovalifolia*, *Olax* sp. and *Anthocleista* sp. also occurred.
- General The riverine vegetation became increasingly wide and dense, reaching up to 50 ft. in depth on either side of the stream, and the canopy was mainly composed of *Syzygium guineense* and *Uapaca somon*.

INSECTICIDE STUDIES ON EAST AFRICAN AGRICULTURAL PESTS.

I.—*EPILACHNA HIRTA* (THNB.).

II.—*CYLAS PUNCTICOLLIS* BOH.

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The practice of intensive farming of European and native crops in East Africa is comparatively recent, and serious attacks there by introduced and indigenous insect pests are therefore a recent development compared with similar outbreaks elsewhere on crops that have been farmed for a greater period. As many of the crops themselves are introductions in a strange environment in territories which were undeveloped 60 years ago, many of the outbreaks are of insects that have not been previously recorded, and that are often unnamed.

In consequence, correspondingly less is known about methods of chemical control under East African conditions, and the present work is one of a series intended to meet the need for published results of experiments with contemporary materials under controlled laboratory and field conditions. The use of some possibly valuable insecticides has been limited in some trials on account of their unsuitability for African conditions, their poisonous nature or their not being available.

In this series, most of the results fall within the first two stages of a three-stage investigation into methods of controlling East African agricultural pests. These three stages are an initial laboratory investigation, small-scale field and plot trials, and large-scale field trials. In the case of the two pests with which the present paper is concerned, the author has not carried work beyond the first stage. The extension of these results to large-scale field application remains to be carried out within the territories as opportunity permits. The use of the larger tractor-drawn and automatic sprayers and dusters waits upon increased financial and technical advancement in many sections of the community.

I. *Epilachna hirta* (Thnb.).

This species of Coccinellid has been recorded as causing injury to a variety of crops in Kenya (Le Pelley, 1952b). Another species, *E. similis* (Thnb.) was first recorded as defoliating *Eleusine*, rice and maize in Uganda in 1932 (Tothill, 1940, p. 147). Damage by *Epilachna* species has also been recorded from Tanganyika, where nine species have so far been identified. In the Arusha district of Tanganyika, during dry weather, maize and potatoes are often severely attacked, causing the leaves to wilt and die. *Epilachna* species also occur in Rhodesia, Australia, India and elsewhere. *E. varivestis* Muls. is the notorious Mexican Bean Beetle in U.S.A. and Canada. Both adults and larvae of *E. hirta* cause characteristic perforating damage to the foliage of potato, maize, wheat, oats, barley, sunflower and cotton (Graham, 1953) and can be present in large numbers over a wide area during an outbreak, as was the case in Kenya in May and June 1950 (Le Pelley, 1952a). Excessive damage to the epidermal layer causes wilting and death of the plants. The insect is normally found in small numbers which only occasionally reach outbreak proportions.

Results of previous work.

No results of experiments with insecticides against *E. hirta* have been recorded,* but much work has been published in North America on *E. varivestis*. DDT and benzene hexachloride (O'Kane, 1947) and other chlorinated hydrocarbons were found to be relatively ineffective, but parathion and HETP (Huckett, 1948; Fjelddalen, 1950) and mixtures of these with rotenone and DDT have given good results. Pyrethrum was also effective (Brannon, 1949). The most effective insecticide, and the only one with an observed ovicidal action is parathion (Norton & Dewey, 1950), although the newer malathion has been reported to give good control (Ginsburg, Filmer & Reed, 1952). The standard recommendation of the United States Department of Agriculture is, at the time of writing, cryolite and rotenone for *E. varivestis* on beans.

Experimental method.

An outbreak of *E. hirta* on wheat allowed a large number of adults to be collected. Sufficient were available for a series of tests in the laboratory to compare three insecticides in dust form.

A sedimentary dusting apparatus was used, in which a weighed quantity of dust is blown upwards into a bell jar by compressed air. The dust then settles for variable times on the insects, which are confined on filter paper. By varying the initial amount of dust and the settling time, different amounts of deposit were obtained. These were checked by weighing. The numbers available allowed five replications of ten adults each per treatment and control. After treatment, the insects were kept in muslin-covered glass tubes at an uncontrolled temperature of about 73°F.

Materials used.

"Agrocide" 3, containing 0.65 per cent. γ BHC.

DDT (technical) dust, locally compounded with diatomite, containing 5 per cent. DDT.

Parathion dust, locally diluted, containing 0.5 per cent. parathion.

Parathion dust, containing 2 per cent. parathion.

Results.

The relationship between insecticide formulation and speed of action is most clearly shown by the time-mortality curves (fig. 1). Speeds of action differed too much for a comparison of median lethal deposits by means of the probit method.

Parathion 2 per cent. dust was the most toxic and rapid insecticide formulation tested, and even parathion 0.5 per cent. dust gave very nearly 100 per cent. kill after 24 hours.

DDT 5 per cent. dust was less rapid in action but produced very good results by the end of 3 days. It offers the possibility of a practical control if the dangerous nature of parathion remains an objection to its use. Benzene hexachloride, as "Agrocide" 3, is relatively less effective, and at the highest rate of application only reached 100 per cent. mortality after eight days.

* Since this work was done, the Colonial Pesticides Research Unit has carried out two trials against an *Epilachna* sp. at Arusha (Progress Report no. 16, 1955). In laboratory trials, DDT gave 100 per cent. kill, both by spraying beetles and leaves at a rate of 50 mg. per sq. ft., the beetles being transferred to clean leaves, and by spraying beetles and leaves at a rate of 5 mg. per sq. ft., the beetles being left in contact with the leaves for 48 hours.

A field trial with DDT, using a Micron Sprayer, showed that the kill fell to 50 per cent. at 20-30 yd. from the sprayer. At this distance, the deposit was about 0.02 mg. DDT per sq. yd. Wind direction was unfavourable.

Extension of results to field conditions.

Until such time as the larger farms possess power-driven drift or row crop dusters, application of DDT dust with rotary hand dusters in the areas of high infestation is to be recommended. In the event of major outbreaks, the communal use of larger dusters might be possible for large acreages. Small areas present no difficulty, provided that the usual safeguards for the application of insecticides to food crops are borne in mind.

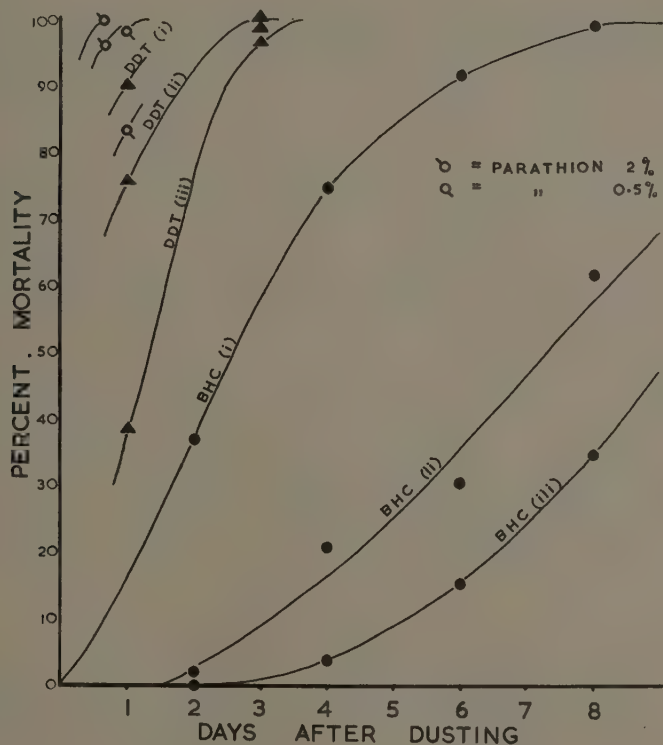


Fig. 1.—The relation between mortality of adults of *Epilachna hirta* and time after being dusted with insecticides. (Corrected for mortality in the controls.)

The deposit rates of active ingredient, in mg./sq. ft. were as follows: BHC (i), 1.04 γ BHC; BHC (ii), 0.52 γ BHC; BHC (iii), 0.23 γ BHC; DDT (i), 5.82 DDT; DDT (ii), 1.66 DDT; DDT (iii), 0.36 DDT; parathion 2%, 2.97 and 0.98; parathion 0.5%, 0.74 and 0.24.

II. *Cylas puncticollis* Boh. A Sweet-potato Weevil.

The sweet potato is an important food crop in many areas of East Africa and is valuable as a famine reserve in some parts. Under good conditions, a yield of up to 20 tons of tubers per acre can be obtained, with an average of about 7 tons (Tothill, 1940). This author (pp. 131-132) also gives an account of the life-history and damage caused by *C. puncticollis* and *C. formicarius* (F.), the two species of sweet-potato weevil common in Uganda. The latter has a very wide distribution. It is a pest of sweet potato in the southern States of the U.S.A.,

where it has been extensively studied. *C. puncticollis* causes severe losses in the Kikuyu districts of Kenya, although no details are available about the relative importance of the two species. Three species of *Cylas* occur in Tanganyika. *Cylas* spp. attack sweet potato in West and Central Africa, Madagascar and Australia (Risbec, 1947). Damage is mostly caused in the field by larvae burrowing in developing and mature tubers, thereby introducing rots and making the tubers inedible. Damage also occurs in store.

Results of previous work.

Work has been done in Hawaii on *C. formicarius elegantulus* (Summers), and has shown the superiority of DDT over other insecticides tested (Sherman, 1951). Further work (Sherman & Mitchell, 1953) confirmed the use of DDT as a dip for cuttings for planting, and as a foliage spray against this form of the insect.

Experimental method.

Adults of *C. puncticollis* were obtained as required from laboratory cultures. The breeding of this species presents no difficulties, adults being confined on sweet potato tubers in cages. Oviposition occurs on the tubers, which are removed to other cages where the next generation emerges.

Five replicates of five insects per replicate were sprayed directly with emulsified solutions of the test insecticides in a Potter tower (Potter, 1952), the insects being exposed in petri dishes. After treatment, the insects were kept in muslin-covered glass tubes at an uncontrolled temperature of about 74°F.

Materials used.

The sprays used were prepared from emulsion concentrates containing 25 per cent. DDT (Didimac miscible liquid), 10 per cent. BHC as lindane (Gammalin liquid concentrate), 21.85 per cent. aldrin in emulsifiable oil, and 18 per cent. dieldrin in emulsifiable oil, respectively. Each concentrate was diluted to give three concentrations of active ingredient: 0.5, 0.1 and 0.02 per cent.

A volume of 2 ml. spray at each concentration was used, at an air pressure of 57 cm. mercury and with a plate gap of 0.6 in. The deposit was found to be 0.813 mg. of spray per sq. cm.

Results.

TABLE I.

Percentage mortalities (moribund and dead) of adults of *C. puncticollis* at different intervals of time after being sprayed, corrected according to Abbott's formula.

Treatment	Concentration of active ingredient (%)	Time interval (hr.)		
		18	30	72
DDT	0.5	100	—	100
	0.1	96	—	100
	0.02	42	—	83
γ BHC ..	0.5	100	—	100
	0.1	92	—	100
	0.02	28	—	34
Aldrin ..	0.5	96	96	100
	0.1	75	84	86
	0.02	22	30	27
Dieldrin ..	0.5	62.5	—	83
	0.1	50	—	57
	0.02	25	—	41
Control (water)	—	4	8	12

The percentage mortality data in Table I have been transformed into probits for the 18 hour count, and plotted, in fig. 2, against log concentrations. The following regression equations have been calculated by the maximum likelihood method, and the respective median lethal concentrations and their error shown.

DDT	$Y = 3.97 + 2.77x$	2.4 ± 0.4 mg./10 ml.
γ BHC	$Y = 3.56 + 2.84x$	3.2 ± 0.2 "
Aldrin	$Y = 3.71 + 1.87x$	4.9 ± 1.1 "
Dieldrin	$Y = 4.18 + 0.71x$	10.4 ± 1.6 "

It will be seen that, in emulsion sprays, DDT, at the 18-hour point, is the most toxic of the insecticides tested, and γ BHC is only slightly inferior.

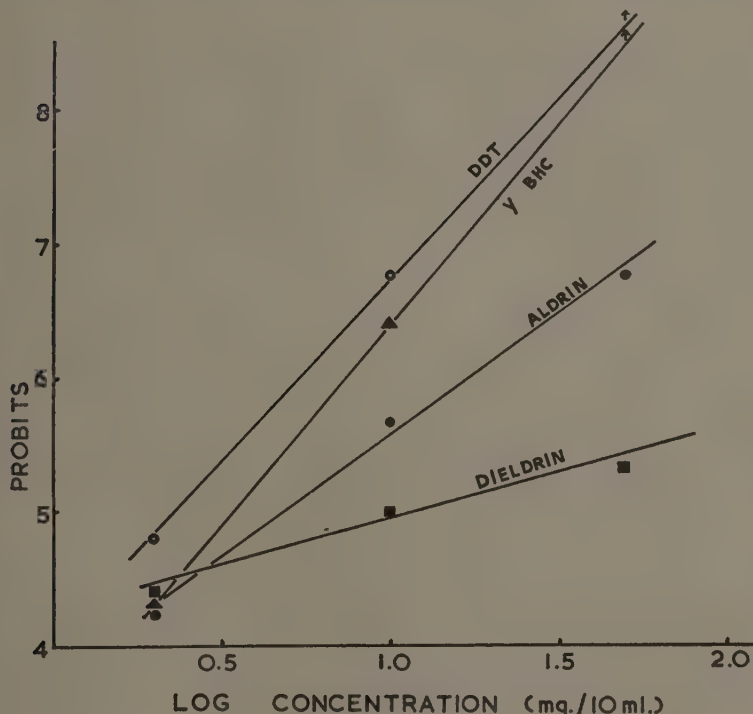


Fig. 2.—*Cylas puncticollis*. Probit regression lines for four insecticides as emulsified solutions, after 18 hours. Corrected according to Abbott's formula.

Extension of results to field conditions.

Propagation in Africa is by the planting of vines or haulms, and infestation occurs from beetles remaining in the soil from a previous crop or from infested vines.

Apart from clean-crop practices and planting in non-infested ground, infestation of the crop can be prevented by dipping or dusting the planting material in insecticide and by spraying the foliage during the season (Sherman & Mitchell, 1953). Adults of *C. puncticollis* spend longer on the foliage than do those of *C. formicarius* and should be more susceptible during this period, although

repeated spraying would be uneconomic on African crops at present. Field experiments with DDT as a spray or dip, or in the dust form, would seem to be worthy of trials in areas where damage is severe.

Summary.

Results are given of laboratory trials of insecticides, carried out in Kenya, against two pests of agricultural crops in East Africa. Against *Epilachna hirta* (Thnb.) (Col., COCCINELLIDAE), the adults and larvae of which feed on the foliage of wheat and other crops, dusts containing 0.5 or 2 per cent. parathion, applied at rates to give 0.74 to 2.97 mg. active ingredient per sq. ft., were the most rapidly effective against the adults. A 5 per cent. DDT dust, at rates giving 0.36 to 5.82 mg. technical DDT per sq. ft., was less rapid in action but produced very good results by the end of three days. A proprietary BHC dust, used at rates giving deposits of from 0.23 to 1.04 mg. γ isomer per sq. ft., was less effective and, at the highest rate used, only reached 100 per cent. mortality after eight days.

Several insecticides were tested in emulsified solutions against *Cylas puncticollis* Boh. (Col., CURCULIONIDAE), one of the important sweet-potato weevils of East Africa. All the insecticides were applied at concentrations of 0.02, 0.1 and 0.5 per cent. active ingredient, and at a deposit rate of 0.813 mg. of spray per sq. cm. DDT was the most effective after 18 hours, but γ BHC was only slightly inferior.

Acknowledgements.

The work was carried out at the Scott Agricultural Laboratory, Nairobi, under the direction of the Senior Entomologist, Dr. R. H. Le Pelley, to whom thanks are due for the provision of laboratory facilities and for much help and advice. Messrs. Shell Chemicals Ltd. and Plant Protection Ltd. kindly provided the insecticides.

Cylas cultures were taken over from Mr. G. M. Lavers, whose subsequent death at the hands of Mau Mau was a great loss.

The work has been financed by the Colonial Development and Welfare Scheme, through the Colonial Pesticides Committee.

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THE BEHAVIOUR OF LARVAE AND PUPAE OF *Aedes aegypti* (L.) IN LIGHT AND TEMPERATURE GRADIENTS.

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Except for some work by Ivanova (1940) very little is known about the behaviour of mosquito larvae in temperature and light gradients.

Ivanova, working with fourth-instar larvae of *Anopheles maculipennis* Mg., found that under the influence of a horizontal temperature gradient, ranging from 37–40°C. at one end to 15–18°C. at the other in a container about 4 ft. long, the majority of larvae congregated in places where the temperature of the water approached an optimum and had no stimulating effect on them. The larvae in the cooler or warmer water were stimulated by temperature and moved about in jerks until they accidentally entered the optimum zone where thermokinesis ceased and they became almost inactive. She suggests that such a distribution of larvae occurs because there is no stimulating effect in the zone of temperature optimum. This results in the aggregation of larvae in this zone.

It is possible that a knowledge of such preferred conditions, for both temperature and light, may be useful in overcoming the difficulties with the species of mosquitos which so far have not been successfully cage-colonised, such as *Anopheles aquasalis* Curry. Two series of experiments were, therefore, carried out to determine the behaviour of larvae and pupae of *Aedes aegypti* (L.) in water, firstly in a temperature gradient at constant illumination, and secondly in a light gradient at constant temperature.

Apparatus and Materials used in Temperature-gradient Experiments.

The apparatus used in this series of experiments, carried out at constant illumination, is shown in fig. 1. It consisted of an aluminium trough, 33½ in. long, the walls of which were about ¼ in. thick. The internal dimensions of the trough, i.e., of the water-containing cavity, were 33 in. long, 1½ in. wide and in. deep. At one end, underneath the trough, was attached a solid cylinder,

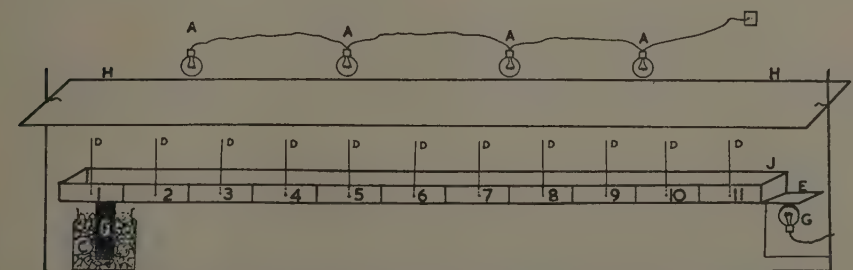


Fig. 1.—Apparatus for temperature-gradient experiments at constant illumination.

- | | |
|--|--------------------------------|
| A. Bulbs for lighting trough. | F. Solid cylinder attachment. |
| C. Bottle containing freezing mixture. | G. Bulb for heating extension. |
| D. Thermometers. | H. Frosted glass sheet. |
| E. Extension of trough for heating. | 1-11. Sections of trough. |

4 in. long and of $1\frac{3}{4}$ in. diameter, of the same material as the trough. This cylindrical attachment was immersed in a freezing mixture of ice and salt. At the other end of the trough there was an extension, under which an electric bulb was placed. By continuous heating of one end of the trough and simultaneous cooling of the other, it was possible to maintain a temperature gradient in the water ranging from 42°C . at one end to 8°C . at the other.

Eleven three-inch sections were marked off along the outside of the trough, and a thermometer, which was not allowed to touch the metal, was suspended with its bulb in the water at the middle of each section. These were used to check the gradient, as well as to record the temperature in each section during the experiment. Convection currents were not of any appreciable strength, judging from the movements of small suspended particles in the water when a gradient was established in the preliminary experiments.

To obtain even lighting along the trough, a plate of frosted glass, 38 in. \times 12 in., was placed above the apparatus (fig. 1) with four 60-watt bulbs connected in parallel series, strung above the glass. The light intensity as measured with an S.E.I. exposure photometer was 0.7 log foot-lamberts. To ensure that no extraneous lighting interfered with the experiments, the entire apparatus was carefully sealed all around with black paper, thereby only letting in light through the frosted glass. A small hole was cut in the paper on one side of the apparatus to permit observation. Precautions were taken to ensure that no light entered through this aperture when readings were being taken. As larvae of *A. aegypti* show an alarm reaction to shadows, etc., it was necessary to remain as still as possible not only while counting larvae but also between the counts.

Larvae and pupae for the experiments were obtained from eggs supplied by the London School of Hygiene and Tropical Medicine, where a permanent standard culture is maintained, and they were reared according to the instructions recommended by the London School.

Method.

Before the beginning of each experiment, it was necessary to set up the temperature gradient in the water, this taking on the average not more than 20 minutes. In each experiment 25 larvae or pupae were used. These were at least a day old in their respective instars, i.e., a fourth-instar larva was used one day after moulting from the third, to allow for hardening of cuticle, etc. The animals were introduced by means of a pipette and spread evenly along the trough, care being taken not to place any in the region above 40°C ., which would kill them, or in the region under 10°C ., in which they became inactive. Animals once used were never used again. Because of their very small size, the first-instar larvae were not tested since they are difficult to see when motionless.

The first reading was taken five minutes after the animals had been introduced, and thereafter every five minutes for 45 minutes. The number of larvae or pupae was counted in each section, the thermometer reading for that section being taken at the same time. Care was taken to see that actively swimming animals were not counted twice.

As the water in the trough was only $\frac{1}{2}$ in. deep, the difference in temperature between the upper and lower surfaces was not great. This shallowness provided very little room for vertical movements of the animals, restricting them to movements either from side to side or from the hot to the cold end. After introduction into the trough, animals generally swam actively, but this initial activity died down soon after they aggregated in the range of temperature where they were least active. Animals immobilised by cold were reactivated on being transferred to warmer water after the end of the experiments. At the hot end, animals quickly showed avoiding reactions, usually swimming back to the cooler regions. Experiments with each instar were repeated ten times.

Controls.

The controls for this series of temperature-gradient experiments also served as controls for the investigation of larval and pupal behaviour in a light gradient. These controls were all carried out in a constant temperature room, maintained at 25°C., in which the temperature of the water remained constant at 23.5°C. The trough was evenly illuminated, the light intensity as measured with an S.E.I. exposure photometer was 0.5 log foot-lamberts. Water for use in the experiments was left overnight in the constant temperature room to reach equilibrium. Twenty five larvae or pupae were used in each of the five control experiments. The animals were distributed along the trough as evenly as possible at the beginning of the experiments and the first count taken after five minutes. Counts were then taken every five minutes for the next 45 minutes.

All the larvae and pupae showed a remarkable similarity in behaviour under these conditions. In the absence of light- and temperature-gradient influences, the animals moved freely along the trough, aggregating at both ends. This "end effect" is clearly shown in the histograms plotted for the total number of larvae and pupae counted in their respective sections (figs. 2 & 3).

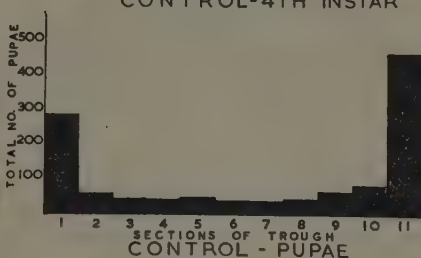
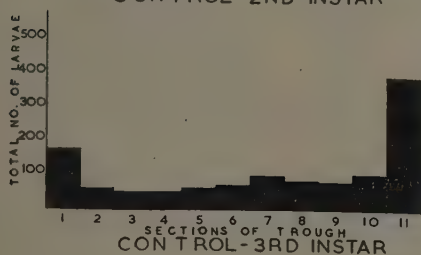
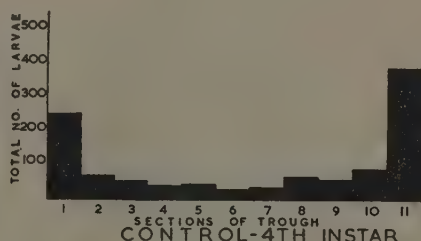
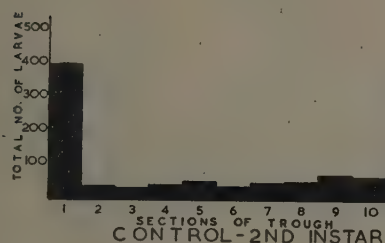


Fig. 2.—The distribution of 2nd- and 3rd-instar larvae in control experiments with water temperature constant at 23.5°C. and light intensity constant at 0.5 log foot-lamberts.

Fig. 3.—The distribution of 4th-instar larvae and pupae in control experiments with water temperature constant at 23.5°C. and light intensity constant at 0.5 log foot-lamberts.

Results.

To complete the results, counts were made in all ten experiments of each series, of the total number of animals settling in the following temperature ranges: 8–12°C., 13–17°C., 18–22°C., 23–27°C., 28–32°C., 33–37°C., 38–42°C. The temperatures were measured in the centre of each of the eleven sections and at each observation the numbers of animals in each of these sections were recorded. The sections were then grouped into seven groups of five-degree intervals of temperature, e.g., the two sections whose centres had measurements of 19 and 22°C. fall in the interval 18–22°C., while the two with central measurements of

24 and 27°C. fall in the interval 23–27°C. So that these grouped figures could be compared with others where the 5°C. interval was represented by one of the original eleven sections, the grouped figures of animals were divided by two, to allow for the larger area. Histograms with these totals plotted against the ranges they represent are shown in figs. 4, 5, 6 and 7.

Second-instar larvae.

From the total number of larvae counted in the respective temperature ranges, it is seen that the majority of larvae were found in the 23–27°C. range (fig. 4), the next zone of preference being between 28 and 32°C.

In the course of the ten experiments, 184 second-instar larvae were immobilised in the 8–12°C. range. Several larvae ventured out into temperatures over 32°C. but the majority remained there only temporarily, returning to the zone of least activity. Second-instar larvae were more active than any other instar.

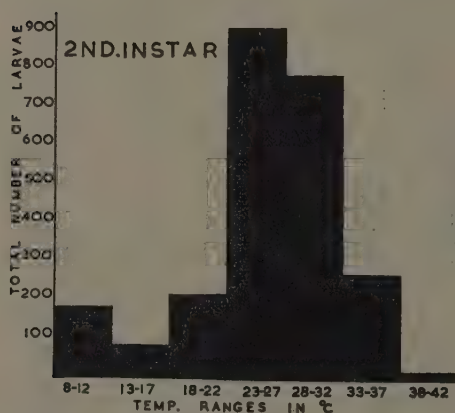


Fig. 4.—The distribution of 2nd-instar larvae in a temperature gradient of 8–42°C. with light intensity constant at 0.7 log foot-lamberts.

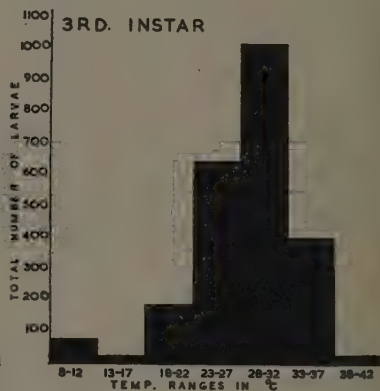


Fig. 5.—The distribution of 3rd-instar larvae in a temperature gradient of 8–42°C. with light intensity constant at 0.7 log foot-lamberts.

Third-instar larvae.

From the histograms (fig. 5) showing totals plotted for the third-instar larvae, it is apparent that there is a marked preference for the temperature range 28–32°C.

Compared with second-instar larvae, there is a shift from the range 23–27°C. as the most preferred zone. The third-instar larvae, however, showed a tolerance for a wider range of temperatures, there being a fairly big total between 33 and 37°C. Again, on either side of these three “optimum” ranges the numbers dropped off considerably. The total number of larvae counted between 18 and 22°C. is not significantly different from the corresponding total for second-instar larvae. Having entered the colder regions of the trough, these larvae were not only capable of remaining active in the low temperature but were also capable of swimming back to the higher temperatures.

Fourth-instar larvae.

Fourth-instar larvae also showed a marked preference for the zone of 28–32°C. (fig. 6). The total number of larvae counted in the range between 23–27°C. and

33–37°C. is considerably lower than the total counted for the range between 28 and 32°C.

Except for the fact that significantly more fourth-instar larvae were trapped in the cold end of the trough, the behaviour of this instar is not very different from that of the third.

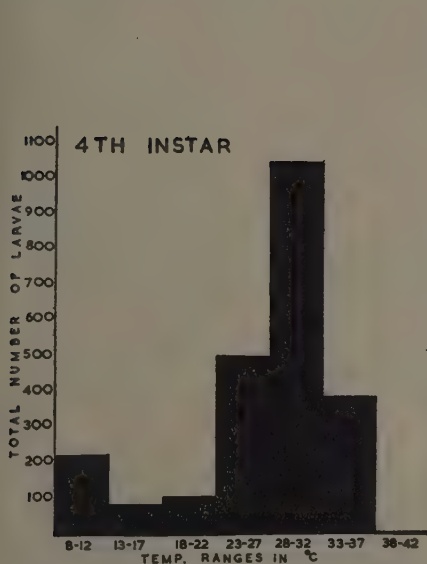


Fig. 6.—The distribution of 4th-instar larvae in a temperature gradient of 8–42°C. with light intensity constant at 0.7 log foot-lamberts.

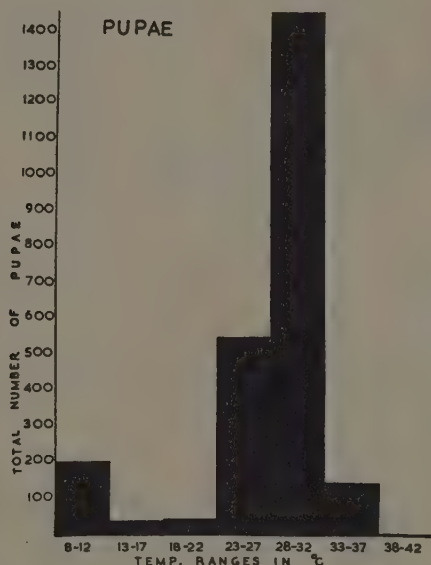


Fig. 7.—The distribution of pupae in a temperature gradient of 8–42°C. with light intensity constant at 0.7 log foot-lamberts.

Pupae.

The preference for the temperature range 28–32°C. is very marked in the pupae (fig. 7), the range with the next highest total being 23–27°C. Both these totals are significantly higher than the corresponding totals for fourth-instar larvae. As in the different larval instars, the numbers drop considerably on either side of these two ranges of preference.

While the experiments were in progress, it was quite common to find as many as 20 of the 25 pupae aggregated in the region of the trough where the water temperature was between 28 and 32°C. The number of pupae becoming immobilised in the cold region is not significantly different from that in the fourth-instar larvae.

The conclusions from these experiments are given in the final discussion.

Apparatus and Materials used in Light-gradient Experiments.

In this series of experiments, the same trough was used as in the experiments with the temperature gradient, and as before, it was marked off into eleven three-inch sections, but, on this occasion at a constant temperature. The trough was set up in a constant temperature room, maintained at 30°C., and a light gradient was established from one end of the trough to the other. This was obtained by

suspending four 100-watt bulbs at one end of the trough; the far end, in the absence of all other sources of light, was thus less strongly illuminated. From the bright end to the dark end there was a gradual decrease in light intensity, forming a gradient in which it was possible to work.

To prevent any heating of the water in the trough by radiation from the bulbs, a glass tank filled with clean water was placed between it and the bulbs. The water for use in the trough was kept in the constant temperature room for at least 24 hours before the experiment commenced to allow enough time for an equilibrium to be reached with the room temperature. Thus, a light gradient was set up from one end of the trough to the other with the temperature of the water constant at 28.5°C.

The light intensities measured by an S.E.I. exposure photometer in log foot-lamberts at the middle of each section were as follows:

Section	1	2	3	4	5	6	7	8	9	10	11
Light intensity (log foot-lamberts)	1.08	0.74	0.40	0.19	0.025	1.96	1.73	1.53	1.40	1.30	1.25

Three thermometers were suspended with their bulbs in the water to see that the temperature of the water remained constant in the trough throughout the experiments and it was found to do so at 28.5°C. All larval instars except the first were used.

Method.

At the start of the experiments, all the lights except those necessary for the experiment itself were turned off. Twenty five animals were then introduced into the water, care being taken to have them spread out as evenly as possible along the trough. Counts were made after the first five minutes, followed by counts every five minutes for 45 minutes and histograms were plotted for the total number of animals counted in each section from all the five experiments.

Results.

Comparing the histograms for this series of experiments (figs. 8 & 9) with those for the controls (figs. 2 & 3) it seems that all the instars used in the experiments react to a light gradient. It has already been observed that, in the absence of any gradient, larvae and pupae swim freely from one end of the trough to the other, and congregate at both ends. Under the influence of a light gradient, however, larvae and pupae display an altogether different pattern of behaviour.

Second-instar larvae.

From the histograms and totals for this instar, there appears to be no marked preference for any one light intensity between the range 1.08 and 1.25 log foot-lamberts. After being introduced into the trough, the larvae initially moved away from the light source but, with time, they started moving slowly towards it. In some cases, when the first count was taken, there were no larvae in the brightest section, but after 30 minutes there were as many as nine in the same section and, as shown in the figure, there is a gradual decrease in the number of larvae in the respective sections as the light intensity decreases. Most of the larvae congregated in the more brightly illuminated sections, and hence showed a slightly photophilic reaction.

Third-instar larvae.

In this series of experiments, the larvae appear to show no marked preference for any one light intensity. At the start of the experiments there was an initial movement away from the light source, but the larvae started moving freely in various directions after 10–15 minutes. In both these respects, third-instar larvae show some similarity in behaviour to those of the second instar.

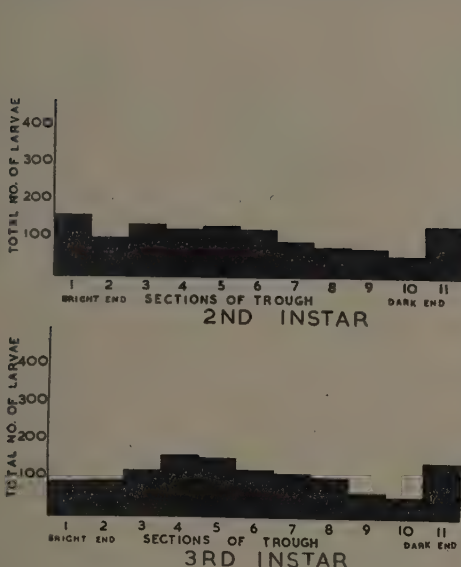


Fig. 8.—The distribution of 2nd- and 3rd-instar larvae in experiments with a light gradient of 1.08–1.25 log foot-lamberts and constant temperature of 28.5°C.

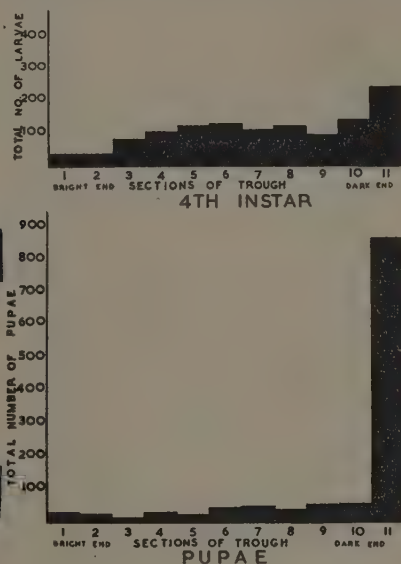


Fig. 9.—The distribution of 4th-instar larvae and pupae in experiments with a light gradient of 1.08–1.25 log foot-lamberts and constant temperature of 28.5°C.

The total number of larvae counted in sections 4 and 5 were slightly higher than in the other sections but from section 6 to section 10 the totals gradually decreased. On taking the regions where the highest totals were counted as being the regions of preferred illumination in this set of experimental conditions, it appears that third-instar larvae show some small preference for light intensity between 0.19 and 0.02 log foot-lamberts.

Fourth-instar larvae.

Fourth-instar larvae behaved differently from those of the second and third instars; they show a negative phototactic reaction as can be seen in the histogram (fig. 9). The total number of larvae increases as the distance from the light source increases, reaching a peak in the darkest section. A total of only 36 larvae was counted in the brightest section in 50 readings as compared with 248 in the darkest section. A few larvae, however, swam slowly towards the middle of the trough after 15–20 minutes.

Pupae.

The experiments with pupae gave more conclusive results than those for the other instars and showed the pupae to be by far the most photophobic (fig. 9).

On being transferred to the trough, at the beginning of each experiment, the majority of pupae not only moved away from the light but remained in the darkest section.

The number of pupae in the darkest section increased with time, and it was not uncommon to find more than 20 of a total of 25 pupae "in hiding" at that end. Few pupae were seen moving towards the light source.

Discussion.

Judging from the results as a whole, it is apparent that second-, third- and fourth-instar larvae as well as pupae of *Aedes aegypti*, when subjected to a temperature gradient, exhibit jerking movements until they finally enter a zone of temperature which has no irritating effect on them. These results are similar to those obtained by Ivanova (1940) for *Anopheles maculipennis*.

It appears, however, that as the larvae progress in age and instar they become progressively more selective than the earlier instars. The second-instar larvae show no signs of being irritated by temperatures between 23 and 32°C. With progressively older instars, however, the range of 28–32°C. becomes more and more pronounced as the zone of preference, the pupae being the most sensitive of all.

Avoidance of the higher lethal temperature, above 40°C., was particularly uniform in all the instars. Only two larvae (in the fourth instar) died on venturing out into the hottest region of the trough.

The pattern of inactivation was very much the same throughout for the instars used, the third-instar larvae showing the greatest capability of swimming back from the cold regions into the optimum zones. That these movements were mainly thermokinetic is at once apparent on comparing the histograms representing the numbers of larvae in the temperature gradient with those in the controls. While the controls show a pronounced "end effect", the temperature-gradient experiments show the reverse.

As larvae of *Aedes aegypti* grow older and change from one instar to the next, they show an increasingly stronger negatively phototactic reaction, this being marked in the fourth-instar larvae and becoming very pronounced in the pupae. It is possible that this behaviour might well be correlated with the development of the compound eye in the larvae. The imaginal eye begins its development alongside the simple eyes in the early instars and reaches an advanced stage in the pupa. Though perhaps non-functional as visual organs, it is likely that, with this development of the imaginal eye, larvae, and eventually pupae, become more and more sensitive to light.

It is known that some adult mosquitos rest during the day-time in shady places, becoming very active only after dusk and during the night. The behaviour of pupae, which congregated in the darkest section of the trough, is therefore in some ways similar to that of the adults, both pupae and adults showing a dislike for light of strong intensity, this probably being a function of the compound eyes.

Summary.

Experiments with second-, third- and fourth-instar larvae of *Aedes aegypti* (L.), as well as pupae, were carried out to determine their behaviour in temperature and light gradients in an experimental trough.

When second-instar larvae are subjected to a temperature gradient from 42°C. at one end of the trough to 8°C. at the other, the majority of larvae aggregate over the range of temperature 23–32°C. Third- and fourth-instar larvae and pupae, however, show a marked preference for the range of temperature between 28–32°C.

When subjected to a light gradient from 1.08 log foot-lamberts to 1.25 log

foot-lamberts, second- and third-instar larvae show no marked preference for any one light intensity. The majority of fourth-instar larvae, however, aggregate in the darkest region of the trough, and this negative phototactic reaction is very pronounced in the pupae. This behaviour is probably correlated with the development of sense organs as the larvae grow. In the case of light gradients, it is likely that the increasing negative phototactic reaction is due to the increasing sensitivity of the imaginal compound eye which starts developing in the early larval instars and reaches an advanced stage in the pupa.

Acknowledgements.

I am indebted to Professor O. W. Richards in whose Department the work was carried out. I am also grateful to Dr. N. Waloff, for her assistance and unfailing interest in the experiments. Dr. H. Leeson, of the London School of Hygiene and Tropical Medicine, was kind enough to supply me with eggs of *Aedes aegypti*.

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THE EFFECTS OF A CHEMICAL DEFOLIANT ON AN ISOLATED TSETSE FLY COMMUNITY AND ITS VEGETATION.

By J. D. FRYER¹, D. L. JOHNS² and D. YEO³

(PLATE VIII.)

Tsetse flies (*Glossina* spp.) depend for their survival upon the shade conditions provided by the woodland or thickets in which they live; removal of this shade by clearing the woody vegetation is a standard method for their control. The possibility of using an arboricide to reduce the shade cast by treated trees was first considered by Swynnerton (1921). His work in East Africa, later followed up by Napier Bax, showed that arsenic pentoxide applied to frill-girdles could so injure the trees that the leaves were shed. Swynnerton (1936) has described an experiment on defoliation by Napier Bax which resulted in a partial evacuation by *Glossina swynnertoni* Aust. of the one square mile of defoliated woodland contiguous to the control area. On the basis of these findings it was claimed that had the treatment been applied on a larger scale the tsetse fly would definitely have been eliminated. The arsenical treatment of the thicket was stated to be "very expensive and generally unsuccessful".

The poison hazards and the difficulties of the large-scale application of arsenic pentoxide did not favour the adoption of this technique. Indeed no further development occurred until after the discovery in 1942 of the new group of herbicides, the substituted phenoxyacetic acids, of which the more active compounds were lethal to some herbaceous species at dosages of 0.25 to 2 lb. per acre. The late Professor P. A. Buxton and Professor G. E. Blackman suggested to the Colonial Office that in woodland infested with tsetse fly these compounds might induce leaf fall, but not the death of trees, at a dose level which would allow economic application by aircraft. After preliminary trials in Kenya (J. P. Glasgow, unpublished reports to the Director, East African Tsetse & Trypanosomiasis Research & Reclamation Organisation, 1949), an aerial spraying trial was carried out in 1950 on an island in Lake Victoria using two of the most promising compounds, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Conclusive results were not obtained from these initial tests, which did, however, indicate that at least temporary defoliation of a wide range of woody species could be obtained, particularly with esters of substituted phenoxyacetic acids applied to the aerial parts of the plants as a spray in diesel oil.

Following up these preliminary trials and as part of a wider study of defoliant and arboricides by the Agricultural Research Council Unit of Experimental Agronomy, K. Holly (Chemical defoliant and arboricides in relation to problems of tsetse-fly control. Colonial Office unpublished report, 1952), working in Tanganyika during 1951, developed and used a micro-technique in the testing of a large range of compounds for their defoliant properties under tropical conditions. He concluded that, from the practical viewpoint, derivatives of

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endoxohydrophthalic acid and the chlorinated phenoxyacetic acids were the most promising compounds.

In addition to this testing work, further aerial spraying trials were carried out at Kikore, Tanganyika, by Holly, working with the East African Tsetse and Trypanosomiasis Research and Reclamation Organisation (E.A.T.T.R.R.O.) and the Colonial Insecticides Research Unit (C.I.R.U.). In these trials the *n*-butyl esters of both 2,4-D and 2,4,5-T were applied in diesel oil at a theoretical rate of application equivalent to 1.05 gallons per acre of oil containing 3 lb. of the compound. With the Anson aircraft flying close to the canopy it was found that only a small proportion of the spray ejected from the Bray O nozzles reached the ground, probably because of unfavourable meteorological conditions (Yeo & Thompson, 1953, 1954). Nevertheless, the butyl ester of 2,4,5-T resulted in a very marked degree of leaf kill or defoliation of many of the important woody species present, including *Isoberlinia globiflora*, *Acacia spirocarpa*, *Commiphora fischeri*, *C. schimperi* and *Lannea humilis*.

In view of the performance of the butyl ester of 2,4,5-T, which was then freely available, it seemed desirable to follow up these trials with applications on a larger scale and to obtain information on the effect of defoliation on an isolated population of tsetse flies. An area of woodland was suggested by Dr. J. P. Glasgow of E.A.T.T.R.R.O., and after a reconnaissance it was decided to carry out an experiment, the principal objects of which would be: (a) to defoliate and maintain in a leafless condition the woodland for a period of not less than three months, (b) to measure the effects of this defoliation on the tsetse fly population, (c) to gain further experience of the effects of aerial applications of 2,4,5-T on the woody plant species and (d) to measure the distribution of the spray droplets in this type of vegetation.

This paper describes the main results of the experiment, which was carried out in 1952 on a peninsula called Waturi on the southern shore of the Kavirondo Gulf of Lake Victoria (lat. 0°28'S., long. 34°17'E.). The project was carried out jointly by E.A.T.T.R.R.O., C.I.R.U. and the Unit of Experimental Agronomy. Funds were provided by a grant from the Colonial Development and Welfare scheme and sanctioned by the Colonial Office Insecticides, Fungicides and Herbicides Committee.

Description of the Area.

The Waturi peninsula is approximately 35 acres in area and rises at its highest point to about 100 ft. above the level of Lake Victoria (3,723 ft. above sea level). The greater part of the vegetation was very dense, consisting of evergreen thicket around the lake shore dominated by large *Ficus* trees and, in the centre, of a mixed evergreen-deciduous thicket that had been intermittently cut over during recent years. The following woody plants were abundant, *Acalypha* spp., *Ficus* sp., *Grewia* sp., *Synadenium grantii*, *Cissus rotundifolia*, *Boscia* sp., *Acacia pennata*, *Lannea stuhlmannii*, *Psiadia arabica*, *Commiphora pilosa*, *Rhus* sp. (probably *natalensis*), *Allophylus* sp., *Lecaniodiscus vaughaniae*, *Euphorbia candelabrum* and *Euphorbia tirucalli*. The vegetation had not been burned within living memory, and before the experiment started was almost impenetrable, the only means of access being along animal tracks. Animals recorded at Waturi were: bushbuck (*Tragelaphus scriptus*), bushpig (*Potamochoerus porcus*), leopard (*Felis pardus*), hippopotamus (*Hippopotamus amphibius*), crocodile (*Crocodilus niloticus*), monitor lizard (*Varanus* sp.) and vervet monkey (*Cercopithecus aethiops*).

At the landward end of the peninsula the thicket ceased, giving way to grassland with occasional *Balanites aegyptiaca* and *E. candelabrum*. The peninsula was thus isolated as a tsetse habitat.

(A general view of the peninsula is shown in Pl. VIII, fig. 1).

Climate.

The seasons in the area of Waturi are usually as follows: long rains, mid-March to end of May; cool season, June to September; short rains, October to mid-December; dry season, mid-December to mid-March.

The most unpredictable of these seasons is the short rains, which has been known to start as early as August and as late as November. It is very unusual for any month to be rainless.

No rainfall figures are available from Waturi but the average rainfall for two nearby places, Sekka, $6\frac{1}{2}$ miles east-south-east of Waturi and Mbita Point, $5\frac{1}{2}$ miles west of Waturi, is shown in Table I.

TABLE I.

Monthly rainfall in inches—mean of readings at
Sekka and Mbita Point.

	1952	1953	1954
January	0.17	1.12	0.55
February	0.73	0.52	1.04
March	2.73	2.57	1.47
April	8.38	4.67	5.91
May	4.76	4.08	
June	2.11	3.20	
July	2.95	2.17	
August	3.39	0.77	
September	3.02	0.48	
October	3.17	2.16	
November	1.51	0.96	
December	0.00	1.27	
Total	32.92	23.97	

Methods of Biological Assessment.

Vegetation assessment.

Before spraying, 50 plants representing 17 species at Waturi were selected and marked by Dr. P. E. Glover of the Department of Veterinary Services, Kenya (then of E.A.T.T.R.R.O.), and the late Dr. C. H. N. Jackson of E.A.T.T.R.R.O. These plants were located near one or other of the fly-paths and were well distributed over the peninsula. Assessments were carried out weekly at first, then monthly and finally quarterly. Estimates of the degree of leaf fall were based on a visual scoring method which took into account not only the quantity of leaves present but also the extent to which those remaining had been affected by the spray treatments.

Tsetse fly assessment.

The main species of tsetse present were *G. pallidipes* Aust. in the central part of the thicket, and *G. palpalis* (R.-D.) (subsp. *fuscipes* Newst.) principally in the lakeshore vegetation. Three African assistants carried out weekly catches on a double octagonal spiral fly-path cut into the thicket; the path was traversed in opposite directions on alternate weeks. All the tsetse flies caught were killed after being recorded.

Control fly-rounds were carried out during the same period on a peninsula 11 miles south-south-east of Waturi called Sikiri, which, although being much larger than Waturi had a similar vegetation and the same species of tsetse fly.

The fly-paths at Waturi were cut 15 yards apart, with sectors 50 yards long, under the direction of J. M. B. Harley, who made estimates of the tsetse populations by means of a marking and recapture technique (Jackson, 1948, 1953) during the four weeks from mid-February 1952 until just before the spraying took place. Harley estimated the population of *G. palpalis* within the marking area to be 1,838 non-teneral* males by the extrapolation method, and 1,768 by the re-capture method. The calculated mean length of life was found to be very short, indicating that, on the average, the flies spent only part of their lives in the main "spiral" area. As only a small part of the fly-round passed through recognisably good *palpalis* habitat, this result was not unexpected.

The estimates for *G. pallidipes* appear to be much more accurate, since this species was concentrated on the higher central region and the spiral should, therefore, have provided a representative sample of the population. The non-teneral male population in the marking area was estimated at 862 by the extrapolation method and 1,062 by the re-capture method. So few females were caught that no estimate of their population was attempted. The area covered by the total fly-round was about 14.5 acres.

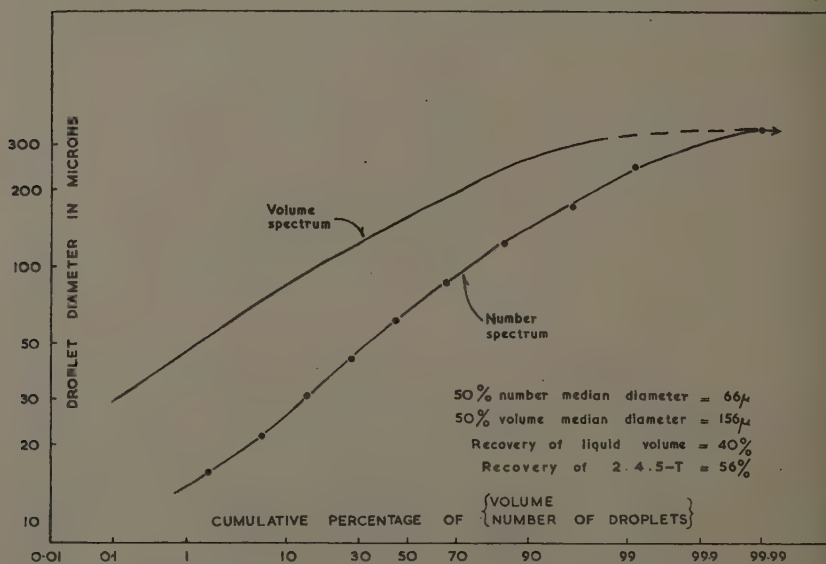


Fig. 1.—The drop spectrum and proportions of the defoliant spray recovered in an open site.

The apparent densities** of the two species during this pre-spraying period were: *G. palpalis*, 171 and *G. pallidipes*, 66. It has been found, in unpublished work, that at Waturi the apparent density of *pallidipes* multiplied by 550 gives an approximate estimate of the population of non-teneral males per square mile.

* Flies with hardened chitin after their first blood meal.

** Apparent density = the number of non-teneral male flies that would have been caught had 10,000 yards of fly-path been traversed.

The Spray Applications.

The Waturi peninsula was sprayed three times at intervals of approximately six weeks using an Avro Anson aircraft fitted with spraying equipment. At each treatment, the *n*-butyl ester of 2,4,5-T was applied in solution in a diesel-oil/aromatic-solvent mixture consisting of 80 per cent. Shell Diesoline and 20 per cent. Petrochemicals aromatic solvent 25-7.

The solution was emitted from a spray boom about 2.5 ft. long, mounted beneath the fuselage of the aircraft and drilled with ten holes, 0.094 inches in diameter and 2.5 inches apart, pointing into the slipstream. A propeller-driven gear pump gave an output from the boom of 12.2 gals. per minute at 105 m.p.h. To achieve the desired dosage a swathe width of 15.5 yards was required.

It was appreciated that the short boom was not ideal; a longer boom would have given a more uniform application but, without adequate check valves, would have led to excessive wastage of solution during the time spent on turning between runs.

Using the same equipment, the drop spectrum had been determined in a separate experiment carried out at an open site, using glass plates coated with magnesium oxide. In this experiment, recovery of 2,4,5-T at ground level had also been determined indirectly by estimating, colorimetrically, deposits on absorbent paper of an oil-soluble dye, Waxoline Red, added to the solution. The details of the drop spectrum and the recoveries in this experiment are given in fig. 1. The volume median diameter* was approximately 160 microns, and the recovery of 2,4,5-T at ground level approximately 60 per cent.

The relevant meteorological and other field data for this experiment are given in Table II.

TABLE II.

Relevant field observations during the determination, in an open site, of the drop spectrum and ground recoveries.

Time (hr.)	Wind speed (f.p.s.) at			Air temp. (°F.) at			Aircraft height (ft.)	Cloud
	8 ft.	16 ft.	32 ft.	8 ft.	16 ft.	32 ft.		
0717-0725	6.8	9.1	10.4	66.7	—	66.6	30	8/8 strato- cumulus

In the applications to the Waturi peninsula, the aircraft was flown close to the top of the canopy. For the first application, on 2nd April, a ground marker was used to indicate the start of each run, which was then completed on a compass course. Subsequent applications were made without markers, the pilot judging the swathe spacings with the aid of a photographic grid of the area. The applications were made as soon as possible after dawn, at a time when the atmospheric turbulence during daylight was minimal. Details of the spray applications are given in Table III.

The meteorological conditions during the first application were close to the condition of dry adiabatic lapse rate. No observations were made on the ground during the other applications, which were, however, carried out during the same period of the day and probably under similar conditions. Relevant field data for

* Volume median diameter = diameter about which the volume is equally distributed, *i.e.*, 50 per cent. of the volume of spray is in droplets less than this size. It equals the mass median diameter if density does not vary with droplet size.

the first application are shown in Table IV. Heavy rain fell overnight before the application and slight rain fell during the early runs. Cloud conditions were 8/8 strato-cumulus during the entire application.

TABLE III.

Details of spray applications.

Application	Date	Concentration of solution (%wt./vol. ester)	Nominal dosage per acre		Actual dosage per acre assuming 60% recovery at ground level (lb. 2, 4, 5-T acid equivalent)
			gallons	lb. 2, 4, 5-T acid equivalent	
1	2.iv.52	37	1.23	3.73	2.24
	7.v.52*	37	1.23	3.73	2.24
2	24.v.52	18.5	1.23	1.87	1.12
3	8.vii.52	10	1.23	1.01	0.60

* A small part of the peninsula that could not be sprayed on 2.iv.52 because of shortage of spray fluid was treated for the first time on this date.

Assessments of the spray deposit at Waturi.

Glass plates coated with magnesium oxide were used for the assessment of droplet distribution and absorbent papers for estimating the recovery of 2,4,5-T. Samples were divided into two groups, those taken on the floor of the woodland or thicket where some of the spray would have been retained by the canopy overhead, and those in the open or upon the top of the canopy. Some comparative figures for the two groups are given in Tables V and VI.

TABLE IV.

Meteorological data for the application on 2.iv.52.

Time (hr.)	Temp. (°F.) at		Wind speed (f.p.s.)	Wind direction towards	Aircraft track towards	Remarks
	2 ft.	6 ft.				
0712	64.1	64.0	4.6	000° M	060° M	1st run
0812	65.5	65.3	5.3	010° M	060° M	last run

The variation in dosage level of 2,4,5-T was in each group approximately thirty-fold. The standard error of the mean in each case was ± 30 per cent. of the average value, a wide variation which was caused by the uneven distribution obtained from the short boom, coupled with the inevitable errors in flying over hilly ground, changes in wind speed and so on.

When the two sample groups are analysed in more detail, they show interesting effects for different droplet sizes. These are summarised in Table VI.

Whereas fewer of the larger droplets were deposited inside the woodland than in open sites, more of the smaller droplets were deposited on the ground inside the canopy. The probable explanation of these results is complex. For coarse aerosols Yeo & Thompson (1954) and Yeo (1954) showed that, in open areas, deposits upon horizontal surfaces were least when the atmospheric turbulence and the wind speed were greatest, and that small droplets, less than 10 microns

in diameter, tended not to be deposited within any reasonable distance from the aircraft. Large droplets, on the other hand, are largely independent of atmospheric eddies and are deposited within relatively short distances from the aircraft regardless of the degree of atmospheric turbulence. Inside a canopy the number of droplets would be attenuated by the vegetation, but the degree of atmospheric turbulence and the wind speed would be less than in open areas, and at Waturi

TABLE V.

Spray deposit data for unshielded and shielded sites.

	Unshielded sites	Shielded sites
(1) % Recovery of 2, 4, 5-T.	49	11
(2) % Recovery of liquid volume	41	6
(3) Volume median diameter of droplets in microns ..	176	156
(4) Number of droplets per sq. cm.	44	19

Note: (1) estimated by colorimetric determination of the dye
Waxoline Red in the spray deposit.
(2), (3) and (4) estimated using magnesium oxide plates.

the increased deposition of small droplets inside the canopy, due to this decreased turbulence and the lower wind speed, outweighed the filtering effect of the vegetation to such an extent that the deposits were actually higher than in the open sites. As the droplet sizes increase, the effect of turbulence becomes less important, and deposits upon the ground within a canopy would be expected to tend to a constant proportion of the deposit in open areas, in fact to a value that

TABLE VI.

Droplet size and density in open and woodland sites.

Droplet size (microns)		No. droplets per sq. cm.		Ratio of no.
Range	Average	Open sites	Woodland sites	woodland/open
10—20 ..	17.5	0.6	1.7	2.63
20—40 ..	29	5.1	6.3	1.24
40—60 ..	50	6.6	4.0	0.61
60—80 ..	69	6.6	2.8	0.43
80—100 ..	90	6.6	1.6	0.24
100—150 ..	123	10.8	1.7	0.16
150—200 ..	168	5.3	0.56	0.11
200—400 ..	235	2.4	0.26	0.11
Totals :		44.0	19.0	

is inversely proportional to the amount of vegetative cover. For droplets greater than 150 microns in diameter the deposits inside the canopy at Waturi were 11 per cent. of the deposits in open areas, and this proportionate value did not decrease with increasing droplet size; this suggests that just under 90 per cent. of the ground was obscured by vegetation, a value that agrees well with our visual estimate of 85–90 per cent. for the amount of vegetative cover.

The leaf washings were recorded in terms of ml. of the original spray solution per square yard of surface, one side of the leaf only being used to measure the surface. With the limited staff and time available, leaf samples from only five plants were taken. The results are given in Table VII—where the leaf deposits are compared with deposits upon nearby horizontal large paper surfaces.

TABLE VII.

Retention of spray solution on leaves and on horizontal paper surfaces.

Sample	Dosage on leaves ml./sq. yd.	Dosage on paper ml./sq. yd.
<i>Acacia pennata</i> ..	0.55	0.08
<i>Acacia pennata</i> ..	0.96	0.94
<i>Ficus</i> sp.	0.85	0.99

The results are few and of the three where comparisons can be made, two suggest that the leaves received dosages comparable with those falling upon large horizontal surfaces, but the third does not. These dosages agree well with those obtained by Holly (Chemical defoliant and arboricides in relation to problems of tsetse-fly control in East Africa. Colonial Office unpublished report, 1952) in the aerial spraying trials at Kikore already mentioned. He found that with a nominal application rate of 1.05 gals. per acre, dosages on leaves of mature trees of *Isoberlinia globiflora* varied from 0.03 ml. per square metre of leaf surface at 10 ft. to 0.12 ml. per square metre at 30 ft. In *Isoberlinia* which had regenerated to a height of 3 ft., he found that the dosage of 2.4,5-T per unit area of leaf surface was very little less than the dosage received on filter papers laid horizontally on the ground.

Experimental Results.

Effect on the vegetation.

Three to four weeks after the first spraying, complete defoliation or death of the leaves had occurred in thirteen out of fifteen species under observation, excluding the succulent and leafless *Euphorbia* spp. By the time of the second spraying, six of these species had produced fresh growth varying from one quarter to complete refoliation. Aerial photographs of the peninsula taken at the time of the first spraying and 25 days later are shown in Plate VIII, fig. 2.

Three weeks after the second spraying, only two species showed signs of producing new leaves. Eleven weeks after the third spraying (*i.e.*, 25 weeks after the first), eight species were leafless and probably dead, five had less than half their normal complement of leaves, and plants of only one species had more than half their normal foliage. The effects of the spray applications on the vegetation are summarised in Table VIII.

From Table VIII it is evident that, taking the vegetation as a whole, defoliation was never complete at any one instant, in spite of all the species being affected at one time or another.

Ten months after the first spraying, Waturi presented a forlorn picture. In place of the initial lush green thicket dominated by large *Ficus* trees, there were now only the leafless skeletons of the *Ficus*, dead *Euphorbia* and moribund bushes. Patches of bright green indicated the presence of *Lecaniodiscus* which had never been seriously affected. Tall grass had grown in the now open areas,

where previously only short stunted grass had been present. It was at this stage that an African child set fire to the grass about a quarter of a mile from Waturi (27.i.53). The fire, after passing through a swampy bay of green *Cyperus papyrus* to the south of Waturi, burned its way through between a third and a half (the southern and eastern parts) of the peninsula. The burnt area was previously of a more open nature than the northern and central parts of the thicket and there were many small grass glades that had been enlarged as a result of defoliation. There is no doubt that the fire was greatly helped by the increased growth of grass and by the large amount of dead wood resulting from the action of the defoliant.

TABLE VIII.

The effect of three applications of the n-butyl ester of 2,4,5-trichlorophenoxyacetic acid on the vegetation at Waturi during the 9 months following the first application.

I. Plants dead 9 months after first spraying.

(a) Rapid defoliation, complete by time of second spraying.

<i>Acacia pennata</i>	<i>Rhus</i> sp.
<i>Cissus rotundifolia</i>	<i>Sesbania</i> sp.
<i>Ficus</i> sp. (reaction of individual trees variable; see note ii below)	<i>Synadenium grantii</i>
<i>Psidium arabica</i>	<i>Vernonia</i> sp.

(b) No defoliation; slow effect resulting in gradual death and final collapse of these species about 9 months after the first spraying.

<i>Euphorbia candelabrum</i>	<i>E. tirucalli</i>
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II. Plants alive 9 months after first spraying.

(a) Rapid defoliation followed by partial refoiliation between first and second, and second and third sprayings; all alive and making strong regrowth 10 months after first spraying.

<i>Acalypha</i> sp.	<i>Grewia</i> sp.
<i>Allophylus</i> sp.	<i>Lannea stuhlmannii</i>
<i>Commiphora pilosa</i>	

(b) Complete defoliation only after third spraying, followed by slow recovery.

Boscia sp.

(c) Partial defoliation; rapid recovery.

Lecaniodiscus vaughaniae

Notes:

- (i) Plants were considered dead when the aerial parts had been killed back completely. It was subsequently observed that *Rhus* sp. and *Acacia pennata* regenerated by means of root suckers.
- (ii) Some trees of *Ficus* sp. were killed; others had branches killed, the trunk remaining alive and subsequently producing new growth.
- (iii) The grasses were unaffected by the spray treatments.
- (iv) The ground succulent, *Sansevieria* sp., was severely checked by the treatment but made rapid recovery.

Effect on the tsetse fly.

The average monthly apparent densities for *G. pallidipes* and *G. palpalis*, together with the number of catches carried out in each month, are given in Table IX.

The defoliation itself had little effect on the apparent density of *G. pallidipes*, although it may have suppressed a seasonal increase at Waturi in the cool season of 1952; such an increase was recorded in June at Sikiri. On the other hand, the decrease in apparent density at Sikiri between June and August 1952 hardly took place at all at Waturi, at a time when the defoliation of leafy plants was maximal. From September 1952 until January 1953 the apparent densities at Waturi and at Sikiri were very similar, November being the only month with any noticeable difference. The effect of the fire in the dry season was almost immediate; by March 1953 the apparent density at Waturi had decreased to about a third of the January figure, while the density at Sikiri had almost doubled. A reduction of

this order might not have occurred had there been no fire. For the last 16 months of the experiment, from March 1953 to June 1954 inclusive, the apparent density at Sikiri was from three to twelve times that at Waturi, the large range in the relative densities being caused mainly by fluctuations at Sikiri. Although *G. pallidipes* was not eliminated at Waturi, it was reduced to a fairly low level, which was maintained for the last 16 months of the experiment with no signs of sustained recovery.

TABLE IX.

The monthly apparent densities of *G. pallidipes* and *G. palpalis* at Waturi and at Sikiri (control).

Month	<i>G. pallidipes</i>				<i>G. palpalis</i>		
	Waturi		Sikiri		Waturi	Sikiri	
	No. of catches per month	Apparent density	No. of catches per month	Apparent density	Apparent density	No. of catches per month	Apparent density
1952							
Jan. ..	—	—	2	79	—	2	62
Feb. ..	17	66*	2	76	188*	2	35
Mar. ..	9	62*	3	14	132*	2	24
	2	138	—	—	90	—	—
Apr. ..	5	123	2	3	134	2	79
May ..	4	149	3	155	105	3	143
June ..	4	143	2	303	70	2	132
July ..	5	109	2	106	33	2	56
Aug. ..	4	164	2	32	23	2	44
Sept. ..	5	170	3	165	23	2	62
Oct. ..	4	191	2	180	51	2	65
Nov. ..	4	167	2	247	70	2	76
Dec. ..	5	228	2	244	54	3	71
1953							
Jan. ..	4	194	2	185	65	2	88
27.i.53 Fire							
Feb. ..	4	110	2	326	43	2	44
Mar. ..	5	75	3	318	58	2	132
Apr. ..	4	89	2	344	78	2	79
May ..	4	67	2	450	100	2	85
June ..	5	86	2	421	169	2	59
July ..	4	39	2	229	93	2	50
Aug. ..	4	64	1	318	97	1	18
Sept. ..	5	55	3	453	109	2	26
Oct. ..	4	47	2	438	116	2	32
Nov. ..	4	54	2	391	92	2	18
Dec. ..	5	86	2	321	115	3	25
1954							
Jan. ..	4	56	2	474	75	2	15
Feb. ..	4	46	2	341	80	2	26
Mar. ..	5	36	3	418	73	2	12
Apr. ..	4	60	1	282	63	1	41
May ..	5	76	2	241	90	1	18
June ..	4	63	2	347	86	2	21

* Supplied by J. M. B. Harley.

The non-teneral female percentage of the non-teneral catch and the teneral percentage for *G. pallidipes* at Waturi, when compared with the results at Sikiri, appear to offer no results of significance relative to defoliation and burning. The

defoliation did not result in any marked increase in the hunger of the population of *G. pallidipes*.

The results for *G. palpalis* at Waturi suggest that the defoliation had some effect between April and May 1952, when the apparent density decreased while that at Sikiri increased. Between May and August 1952 the density at Waturi decreased relatively more than it did at Sikiri. During the same period *G. palpalis*, which was frequently captured in the central part of Waturi during the first few months of the experiment, almost completely disappeared from this region. It seems possible, therefore, that the decrease in density in the fly-round area may have been contributed to by a redistribution of *G. palpalis*, although the seasonal drop illustrated in the control would account for the greater part of the decrease. More direct evidence of such a redistribution, in the form of an increase in the catch of *G. palpalis* along the few sectors of the fly-round that passed through its lakeside habitat, would not be expected at this time because of the appreciable decrease in density that took place at Sikiri. Defoliation progressed more rapidly on the higher, central "backbone" of Waturi than it did near the lake, where *Lecaniodiscus*, the dominant tree, was hardly affected. The damage done by the fire was almost all in the habitat of *G. pallidipes*, the lake-shore habitat of *G. palpalis* being little affected. As previously indicated, a good sample of the population of *G. palpalis* was not obtained from the spiral fly-round; this has reduced the value of these results.

Effect on light intensity.

In order to obtain information on the effects of defoliation on light intensity at ground level, readings were taken with an apparatus incorporating a standard Weston exposure meter under each marked plant at the same time as the vegetation assessments. Before spraying, the level of shade in the thicket under representative plants varied from 0.05 to 0.4 of daylight (full daylight = 1). Following defoliation, according to the reaction of the various species, the light intensity increased to 0.6–0.9 in the case of those broad-leaved species that were completely defoliated. This considerable range was determined by the varying shade caused by the pattern of twigs and branches of plants composing the different communities.

The large number of results obtained are not presented in this paper as they are unlikely to be of general interest and because certain criticisms can be made against the technique employed. In most, but not all, instances the readings are consistent with observations on the effect of the spray treatments on the canopy. The difficulties of obtaining more accurate measurements of light intensity in woodland have been fully discussed by Atkins, Poole & Stanbury (1937) and by Blackman & Rutter (1946).

TABLE X.

Seasonal humidity; the average of six sites on Waturi, expressed as saturation deficit.

Season	1952	1953	1954
Dry season	21	21 (Fire)	17
Long rains	12	14	15
Cool season	14	15	—
Short rains	13	18	—

Effect on humidity.

The average seasonal humidity, expressed as saturation deficit and calculated from readings taken with a whirling hygrometer at six sites on Waturi, is shown in Table X.

Defoliation occurred in the long rains, the cool season and in the short rains of 1952 which are all shown to be humid seasons by these results. No direct correlation between defoliation and humidity can be observed from the available data.

Discussion.

When the Waturi experiment was being planned, the spray applications were aimed at giving maximum defoliation over a period of about three months and dosages were not restricted because of economic considerations. From the information available at that time, the dosages actually applied appeared to be about those that were likely to give the desired effects on the vegetation.

The effects were, in fact, more drastic than envisaged, and defoliation was followed by death of the aerial parts of many plants, suggesting that lower dosages or fewer applications would still have given good defoliation. Recent trials by Ivens (1954) on evergreen sub-riverine thicket and on deciduous thorn bush in East Africa have shown that defoliation of a considerable number of tree species can follow a single aerial application of less than 1 lb. of 2,4,5-T per acre, and that a leafless condition of certain species can be maintained for a period as long as 25 weeks. The results at Waturi and those of Ivens make it clear that leaf fall following applications of 2,4,5-T is likely to take place at varying intervals after spraying, according to the species, and that some species may not be defoliated at all. While other defoliant may be more effective in this respect, it appears that 2,4,5-T is unlikely to bring about complete defoliation of all species and individuals at any single time after spraying, an essential requirement if eradication of *Glossina* is to be achieved through defoliation alone. At Waturi, for example, whilst only *Lecaniodiscus* amongst the leafy species resisted defoliation, it was one of the locally dominant trees. During the time when the trees were shedding or regenerating leaves the two leafless *Euphorbia* species were still little affected. Since they too were abundant on parts of the peninsula, it was estimated that for the first six months, in spite of defoliation, about half of the original tsetse habitat still provided suitable shade for *Glossina*. After the first six months, when the *Euphorbia* species were collapsing, much of the leafy vegetation had put out new leaves. There was, in fact, a suitable habitat available for both species of *Glossina* all the time, although it was not constant in position.

Although, under the conditions at Waturi, defoliation by means of 2,4,5-T failed to eliminate a tsetse fly population following on the removal of shade, the trial has yielded results, which help to clarify the many factors involved. Some of these factors may apply only to the conditions prevailing in bush and climate similar to those at Waturi. Others such as the varying response of different plant species to the defoliant are likely to be important in any mixed community.

One important factor contributing to the small effect of defoliation on the two tsetse species was the timing of the treatments, which took place at the most humid time of the year (Table X). It is known that the death-rate of other tsetse species is higher in dry than in humid air (Jackson, 1940, 1944), and in fact a large proportion of the fall in numbers of *G. pallidipes* took place during the dry season, December 1952 to March 1953. The extension of the defoliation into the cool season would have little effect because it is known that *G. pallidipes* leaves its thicket home in large numbers at this time of the year and exists in more open country.

Another feature at Waturi, not always present in other tsetse habitats, was

the amount of shade cast by twigs and stems in the very dense thicket. Even if complete defoliation of all species had been obtained, this shade might have been sufficient to support the tsetse population.

While the value of defoliation alone as a control measure for tsetse fly has not been demonstrated, the Waturi experiment has shown that under some circumstances eradication of normally fire-resistant bush might be achieved by a controlled burn following a defoliant application. However, it must be reported that a second burn, which was attempted in January 1954, was unsuccessful in spite of favourable conditions, indicating the difficulties that may be encountered if further trials are attempted in which defoliation is combined with burning. The main reasons for the lack of success at Waturi were: firstly, the growth of the grass in the area affected by the 1953 fire had been very scanty, mainly because the short rains of 1953 had failed. Secondly, *Sansevieria* and other ground succulents, untouched by the previous fire, were left as a protective fringe around the unburned thicket and also occurred abundantly within the thicket where they markedly hindered the spread of fire at ground level. Hardly any thicket that did not contain succulents had been left by the fire of 1953 and, in spite of repeated attempts, the remaining thicket could not be made to burn. Lastly, the main species of the thicket had produced a considerable amount of new growth since the first fire and were much greener and less inflammable.

Following up these preliminary indications from the Waturi trial, Ivens has confirmed that the success of this technique is likely to be greatly influenced by the rainfall during the period of defoliation, through its effect on the growth of grass. With a low rainfall there may not be enough growth, however complete the defoliation, to allow a sufficiently fierce burn in the following dry season to destroy the woody vegetation. Another factor is the amount of dead brushwood contributing to the fire. At Waturi the fire was undoubtedly aided by the large quantity of dead inflammable material resulting from the relatively large dosages of 2,4,5-T applied. Smaller dosages likely to be required in practice for economic reasons might not, although possibly causing adequate defoliation, kill sufficient of the woody growth to allow a useful burn to take place.

Under conditions similar to those at Waturi, treatments with a defoliant might be more successful if carried out in the following way. The first treatment should take place in the short rains with the plants in full leaf and some effect on the tsetse might be expected during the subsequent dry season. The treatment would slowly kill the *Euphorbia* which would be in a condition to burn readily by the dry season of the following year. It would also result in much dead wood and would encourage the growth of grasses within the thicket. The second spraying would follow in the short rains a year after the first, the aim being to defoliate regenerating shoots that would be appearing by this time. It would also enhance the effect of the dry season on the tsetse until the time for burning had arrived. The burn would take place as soon as the thicket was sufficiently dry in the second dry season after the first application. Thus, maximum defoliation resulting from the burn would coincide with a considerable period of the dry season and so produce the most unfavourable conditions for the fly population.

Considering the technique of spraying, a more uniform distribution might have been obtained if a wider boom had been used. It is unlikely, however, that this modification would have markedly affected the overall biological results, which were largely dependent upon the fundamental differences in reaction to the spray of the many plant species which formed the tsetse habitat at Waturi.

While 2,4,5-T was selected for the Waturi experiment as the most active defoliant available at that time, a number of other efficient defoliants are known and the possibility that some of them may be more effective than 2,4,5-T under East African conditions is being studied.

Summary.

Three aerial applications of the n-butyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in diesel oil were made at intervals of six weeks to an isolated woodland community on a peninsula in Lake Victoria, Kenya. The woodland contained two species of tsetse fly, *Glossina pallidipes* Aust. and *G. palpalis* (R.-D.) (subsp. *fuscipes* Newst.). The object of the treatment was to defoliate the woodland in order to ascertain whether the reduction in shade would control the tsetse fly. It was also desired to obtain further information on the effect of the spray applications on the woody plant species.

The first application, at an estimated actual dosage of 2.2 lb. 2,4,5-T per acre, resulted in a rapid defoliation of 13 out of the 15 observed leafy species, eight of which subsequently died during the following nine months. Two succulent species of *Euphorbia* also died during this period. Of the remaining two species, one was fully defoliated only after the third application but recovered slowly, while the other, *Lecaniodiscus vaughaniae*, was but slightly affected at any time.

No significant reduction in the numbers of tsetse fly was observed during the nine-month period following the first spraying. The following factors probably account for this lack of effect: the varying rate of defoliation of the different plant species, the resistance to the spray of one locally abundant species, the persistent shade cast by the stems of the thick vegetation after defoliation and the relatively humid climate during the period of maximum defoliation. These factors allowed an environment favourable for the tsetse fly to exist in one part or another of the peninsula in spite of the severe effects on the vegetation.

Nine months after the first spraying, between a third and a half of the vegetation was destroyed during the dry season by an accidental fire. The effect on *G. pallidipes* was almost immediate, and two months later the population had been reduced to a fairly low level, which was maintained for the last 16 months of the experiment. The fire hardly affected the environment of *G. palpalis* and the main effect of defoliation was to cause this species to concentrate near the shady lake shore, where the dominant tree was *Lecaniodiscus vaughaniae*.

There is little doubt that the destruction of the vegetation by fire was made possible by the effects of the spray applications, as the hitherto evergreen thicket would normally have been unburnable. While this suggests a possible new technique for bush clearance, further trials have indicated several factors which may make difficult a satisfactory burn following defoliation.

Details are given of the assessment of the spray droplet performance both in calibration trials and during the applications to the peninsula.

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The African assistants and Mr. K. Wiggwah deserve special mention for their good work at Waturi under difficult conditions.

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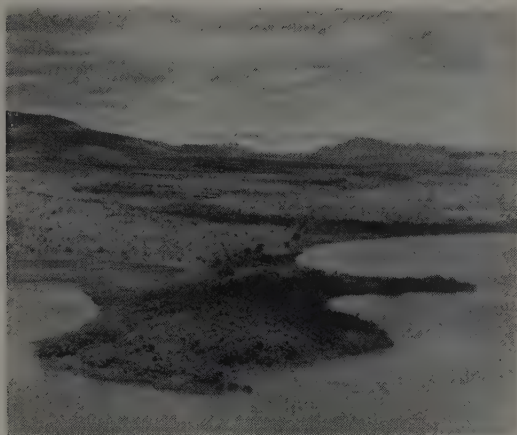


FIG. 1. General view of Waturi peninsula looking west, showing high ridge down the "backbone" with steep banks down to the lake on the north, and papyrus swamps to the south. The isolation of the peninsula as a tsetse fly habitat is well shown.



FIG. 2. Aerial views of Waturi; A, at the time of the first spraying and B, 25 days later. In A, the fly-round paths can just be distinguished at a few points. In B, after defoliation, they are clearly visible. The marked "opening up" of the vegetation as a result of the first spraying is well illustrated.

AN INVESTIGATION INTO THE EFFECT OF CULTURAL CONDITIONS ON POPULATIONS OF THE VECTORS OF VIRUS DISEASES OF CACAO IN GHANA WITH AN EVALUATION OF SEASONAL POPULATION TRENDS.

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A previous study of the ecology of the mealybug vectors of the viruses causing swollen-shoot disease of cacao in Ghana (Strickland, 1951b) showed that populations of the dominant vector, *Pseudococcus njalensis* Laing, vary greatly within relatively small areas of uniform cacao. Samples then taken over a period of two years indicated no major changes in vector population from month to month. The objects of the present work were to determine whether mealybug density is influenced by cultural practice, and to make a more critical study of changes in mealybug population by taking fortnightly samples over a period of 13 months. At the same time, a study was made of the major parasites and predators and the dominant coccidophilic ants, and of the association of these insects with the vector populations.

When collating the results it became evident that the statistical treatment of the field data needed special consideration, and this aspect of the work has been treated in some detail.

Experimental Methods.

Site.

Mealybug reproduction and survival were studied on cacao growing in two contrasted habitats, both typical of large areas of the cacao belt of Ghana. One habitat comprised well-maintained cacao, irregularly spaced and with a closed canopy, which inhibits the growth of ground vegetation and allows free air-movement at trunk level. The second habitat comprised cacao growing in dense secondary bush, simulating the conditions found in farms where the cacao has been severely attacked by Mirids and bush has been allowed to grow to assist in the regeneration of the cacao (Williams, G., 1953). Such bush is composed of mixed herbaceous species interwoven with creepers, producing etiolated cacao and a still and almost saturated atmosphere. About 55 per cent. of individuals comprising the population of wild plants growing in association with cacao are infested by PSEUDOCOCCIDAE, including 17 per cent. that are infested by *P. njalensis* (Strickland, 1951a). An important distinction, therefore, between the two habitats is the infestation of many of the plants comprising the bush vegetation, whereas in the well-maintained cacao the forest trees alone provide additional hosts. A study of mealybug populations was made in eighteen plots, each composed of two adjacent sub-plots, termed the cultivated and the bush sub-plots, representative, respectively, of the habitats described. The plots were situated at Tafo within the same square mile of cacao surveyed by Strickland (1951b) (fig. 1).

Population sample.

The effect of the environments of the two habitats on populations of mealybugs and associated ants, predators and parasites was determined from a sample of

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29 cacao trees selected at random in each sub-plot. The sampling technique employed was that described by Strickland (1951b), which involves felling the trees to allow accurate counting of the vectors. The method is open to the criticism that progressive sampling may change the environment of the plots. In the cultivated areas, not more than a quarter of the trees were used, but, by the end of the survey, the sample taken in some bush sub-plots included almost every cacao tree. The bush vegetation was maintained, however, so that in both habitats infested hosts remained at the end of the survey. The survey was carried



Fig. 1.—The distribution of plots (circles) in relation to the areas (black rectangles) surveyed by Strickland (1951b). The unshaded areas represent cacao available for study. The black and white halves of the circles indicate the relative positions of the bush and cultivated sub-plots, respectively.

out between 5th January 1954 and 11th February 1955 and comprised 29 fortnightly periods, each containing nine working days. Four trees were felled and examined on each working day, providing a sample of one tree in each sub-plot during each fortnight. The plots were assigned at random to working days and thereafter visited systematically. The four cacao trees comprising the sample for any one day were examined at 2 p.m. on the previous afternoon, when a 15-minute count was made of the number of ants of the genera *Crematogaster* and *Pheidole* seen moving on the trunks. Ants are most active at this time of day and their numbers remain constant for a considerable period. After felling, the trees were thoroughly examined in the field, using a binocular microscope. The numbers of mealybug adults and nymphs in each colony were recorded separately, but no distinction was made between mealybug species. Adult mealybugs from each tree were removed to the laboratory for parasite rearing. Any Cecidomyiid or Coccinellid larvae found in mealybug colonies were also recorded.

Climatic factors.

Detailed meteorological measurements were not taken within the plots. Fortnightly means for precipitation and numbers of wet days were calculated from

three rain gauges situated in clearings, two of which were within the survey area and the third half a mile away. Changes in precipitation and numbers of wet days are shown in fig. 2. Using Livingstone atmometers, the evaporation at canopy level was found to be 10 per cent. less under bush conditions than in an adjacent cultivated area. The mean daily saturation deficit recorded at 3 p.m.

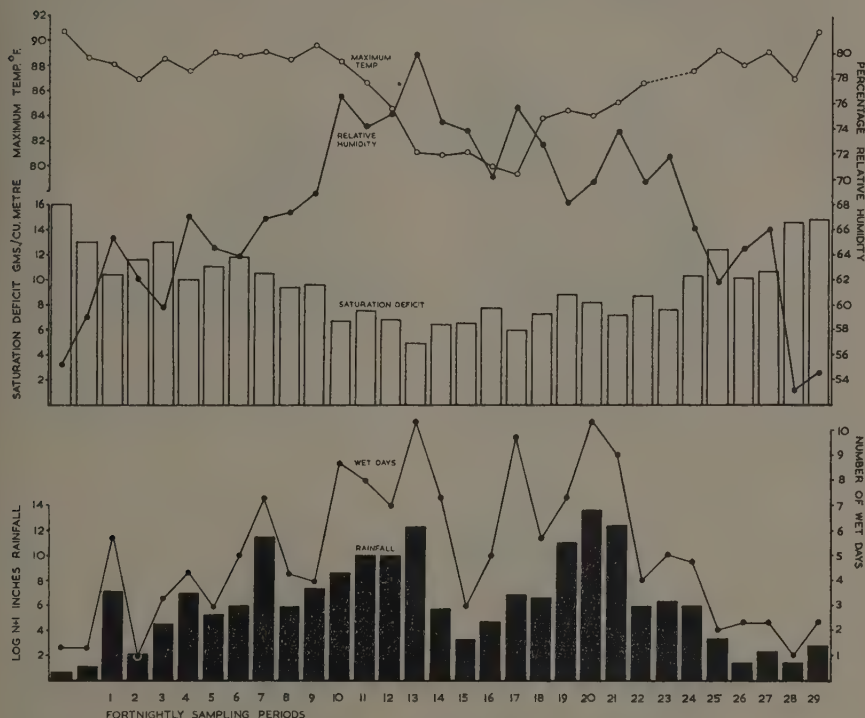


Fig. 2.—Meteorological data.

in a Stevenson screen, half a mile outside the survey area, was found to provide a very good indication of changes in evaporation when compared with atmometers in the field. Changes in saturation deficit and relative humidity recorded in the screen are shown. Using Cambridge continuous recording thermometers, no marked temperature differences between the two habitats were obtained at six feet above the ground. Changes in maximum temperature are shown in fig. 2; the mean minimum temperature fluctuated between 68 and 70°F. throughout the year.

Results of the Survey.

Statistical treatment of the field data.

Frequency distributions of the field data showed that in most cases some form of transformation was necessary before statistical analyses could be attempted. The numbers of sub-plots containing different numbers of trees infested with mealybugs and ants are distributed skewly, but more normal distributions are obtained after square-root transformation (fig. 3 (a) & (c)). The numbers of

sampling periods containing different numbers of trees supporting bugs and ants are distributed normally (fig. 3 (b) & (d)), providing evidence that the plots were sampled in a random manner. The number of infested trees in each sample was

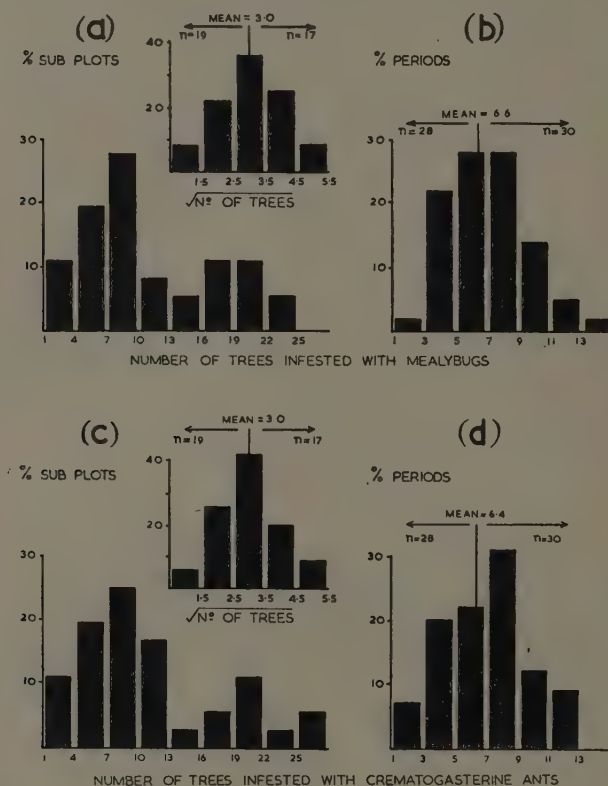


Fig. 3.—(a, b) Frequency distributions of sub-plots (a) or sampling periods (b) containing different numbers of cacao trees infested with mealybugs. (c, d) The same, for ants.

expressed as a percentage and then subjected to the angular transformation of Cochran (1938). The percentage of trees infested has been referred to as the "percentage infestation rate".

Populations of mealybugs on trees (fig. 4 (a)), of mealybugs in colonies (fig. 4 (b)) and of colonies on trees (fig. 4 (c)) are all distributed skewly. Similar distributions were obtained for ants (fig. 5), and for predators and parasites (fig. 6). These data were subjected to the $\log(n+1)$ transformation, allowing comparisons between "geometric" means and totals, which provide more reliable measures of insect abundance than do untransformed data (Williams, C. B., 1937, 1953; Cornwell, 1953). Slight deviations from normality of the transformed data are shown by unequal numbers of observations above and below the geometric means.

The very skew distribution of the data for parasites and predators is not fully corrected by the log ($n+1$) transformation (fig. 6). This may be a result of the association of these insects with a host population that is itself distributed skewly. It was considered that in all cases sufficiently normal distributions of the data were obtained, after using these transformations, to allow the use of conventional statistical techniques.

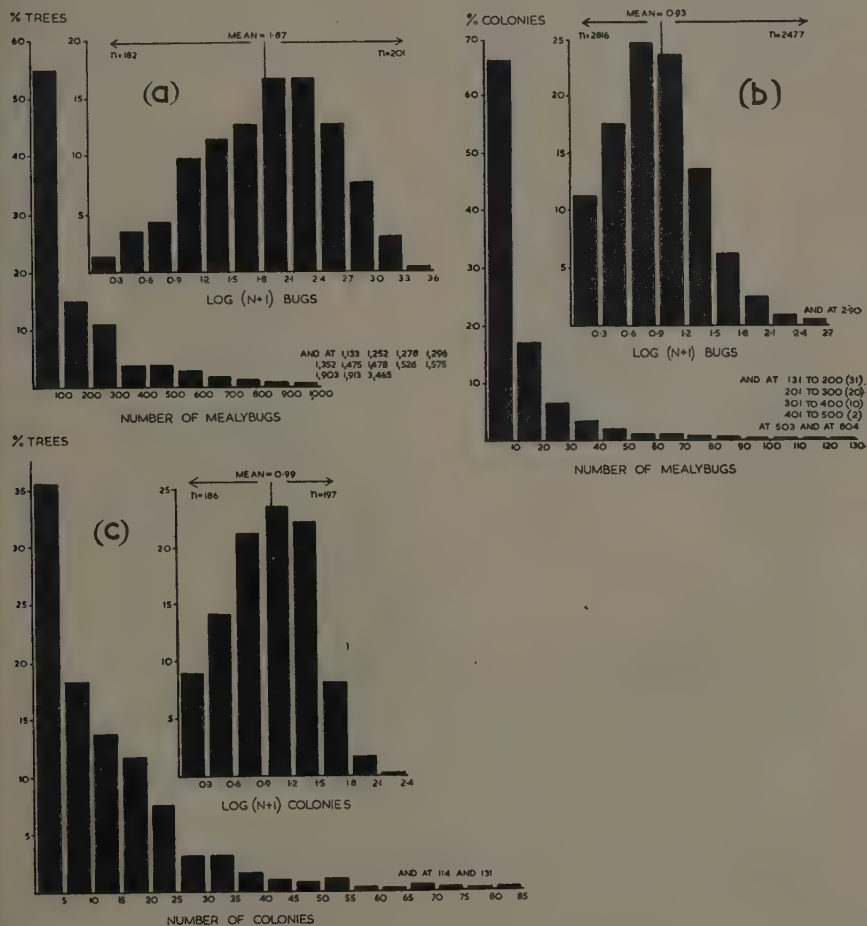


Fig. 4.—Frequency distributions of mealybug populations: (a) trees supporting different numbers of mealybugs; (b) colonies containing different numbers of mealybugs; (c) trees supporting different numbers of mealybug colonies.

The effect of cultural conditions.

The numbers of cacao trees infested by mealybugs, and the populations of these on cacao trees in the bush sub-plots and the cultivated ones, are shown in Table I. The data show the effect of the log ($n+1$) transformation on the relative

sizes of the sub-plot populations. In the bush habitat, plot 11 has the highest arithmetic total population (over 8,000 mealybugs), but a lower geometric total than plot 6. This is due to an extreme tree-to-tree variation in the numbers of mealybugs, three trees in plot 11 each supporting more than 1,000 bugs.

The results suggest that more cacao trees are infested with mealybugs and there are more colonies and higher populations in the bush than in the cultivated areas. However, statistical analysis of the data shows that these differences are not significant. The lack of significance is not surprising in view of the marked variation between sub-plots. Thus, the number of trees infested varies from 1-24 in the cultivated sub-plots, and 2-23 in the bush ones.

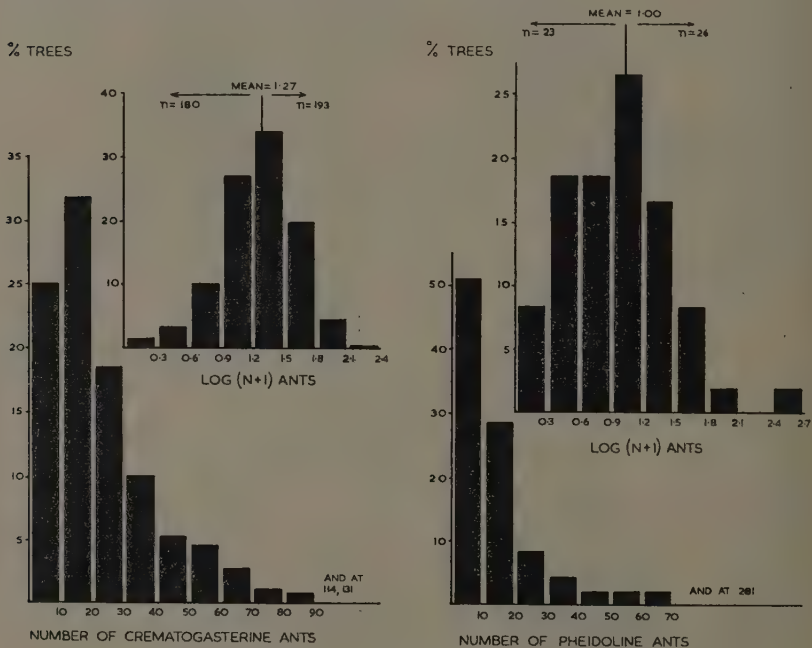


Fig. 5.—Frequency distributions of trees supporting different numbers of ants.

Table II shows that there is a statistically significant difference between the two habitats as regards the number of trees infested with ants. In the bush sub-plots, more trees are infested with ants of the genus *Crematogaster* and populations are higher than in the cultivated areas. The reverse is true of ants of the genus *Pheidole*, which are, however, far less numerous than *Crematogaster* on cacao. The numbers of predators and parasites recorded during the survey are also shown in Table II, together with specific identifications where these have been possible. The numbers of predators indicate only the relative abundance of the populations, since early-stage Coccinellid larvae are difficult to distinguish in colonies containing large numbers of nymphal mealybugs, and CECIDOMYIIDAE spend only part of their larval life feeding externally. Table II shows that Coccinellid larvae are significantly more abundant in the bush sub-plots than in the cultivated ones. Lack of data prevented the statistical examination of the effect of the two habitats on populations of CECIDOMYIIDAE and the various

Hymenopterous parasites. Cecidomyiids are apparently more numerous in the cultivated areas. The most numerous parasites are of two genera, *Anagyrus* and *Clausenia*, which are apparently more abundant in cultivated and in bush conditions, respectively. Apart from these two genera, only two parasites were

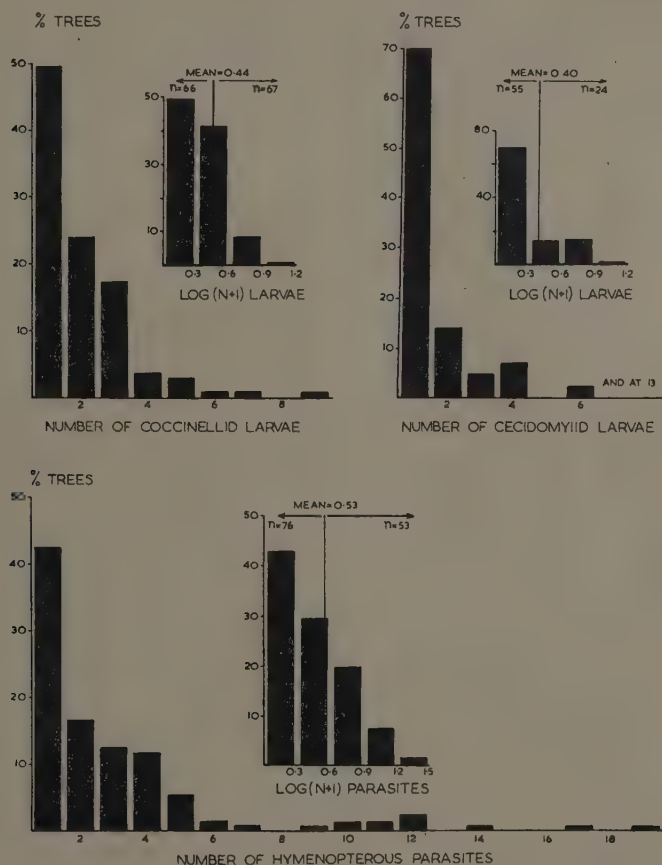


Fig. 6.—Frequency distributions of trees supporting different numbers of parasites or predators.

recorded from the bush sub-plots, compared with 39 from the cultivated ones. There is evidence, therefore, that populations of parasites are more diverse in areas of well-maintained cacao than in the bush.

Biological relationships.

In addition to the comparisons made in the previous section, an examination was made of the relationships between populations and infestation rates of mealybugs, ants, parasites and predators under the two cultural conditions. From the untransformed sub-plot totals in Table I it may be shown that the relation between the number of mealybugs, or mealybug colonies, and the number of infested trees, is of an exponential form. Furthermore, the number of bugs

TABLE I.
Mealybug infestation rate and population on cacao trees in bush and cultivated conditions.

Plot	Bush sub-plots				N	Cultivated sub-plots				
	Number of mealybugs		Number of colonies			N	Number of mealybugs		Number of colonies	
	Arithmetic total	Geometric total	Arithmetic total	Geometric total			Arithmetic total	Geometric total	Arithmetic total	Geometric total
1	9	5,568	20.07	141	9.05	9	1,181	16.34	64	7.35
2	4	587	5.94	46	3.52	5	270	7.51	33	3.90
3	17	2,303	30.73	157	15.53	24	4,466	44.09	264	23.24
4	20	3,671	39.94	387	22.12	14	2,633	25.64	230	14.37
5	5	90	5.99	15	2.76	2	25	2.23	3	0.78
6	23	4,935	48.72	389	26.74	18	3,450	37.14	246	19.88
7	6	207	8.12	25	4.14	9	319	11.33	36	5.45
8	2	255	3.58	28	2.16	17	4,013	36.59	376	20.99
9	10	2,689	22.99	169	12.01	9	1,286	15.42	101	8.55
10	20	5,353	42.94	279	21.71	7	609	10.96	50	5.59
11	21	8,320	45.23	424	23.98	9	671	12.83	38	5.95
12	18	2,175	31.96	220	17.74	7	165	8.44	27	4.44
13	15	1,583	26.26	163	14.36	19	9,964	44.98	675	26.13
14	3	93	3.96	14	1.94	11	3,338	20.94	135	10.34
15	5	2,089	11.10	87	5.50	4	31	2.69	9	1.75
16	5	1,335	11.50	125	6.80	7	825	13.00	93	7.24
17	9	1,954	17.09	92	7.67	1	2	0.48	2	0.48
18	9	761	14.32	66	7.18	10	1,122	18.05	84	8.72
Total	201	43,868	390.44	2,827	204.91	182	34,370	328.66	2,466	175.15

N = Number of cacao trees infested out of 29.

Arithmetic total = Σn , geometric total = $\Sigma \log(n+1)$, where n_1, n_2, \dots = no. of mealybugs (or colonies) in sampling periods 1, 2, . . . etc.

shows a similar relationship to the number of colonies, when the distribution of either one of these factors is rendered normal by transformation. To compare the relationship between mealybug infestation rate and population under bush and cultivated conditions, regression analyses were carried out on the sub-plot totals of the transformed data between pairs of the following factors: number of trees infested, number of colonies and total population. From these analyses, straight-line relationships between the paired variables were obtained, the correlation coefficients measuring the degree of linear association between the factors, and the regression coefficients the slope of the line.

TABLE II.

Populations of ants, predators and parasites.

	Observation	Habitat		S.E.	P
		Bush	Cultivated		
<i>Crematogaster</i>	Total number of trees infested ..	222	151		
	√ Mean no. of trees infested per plot ..	3.36	2.73	±0.19	<5
	Total ant population	5,613	3,237		
	Mean log (n + 1) ants per plot ..	16.06	10.32	±1.69	<5
<i>Pheidole</i>	Total number of trees infested ..	14	35		
	√ Mean no. of trees infested per plot ..	0.68	1.29	±0.16	<2
	Total ant population	120	796		
	Mean log (n + 1) ants per plot ..	0.71	2.01	±0.31	<1
Predators	Total population of Coccinellid larvae (<i>Platynaspis</i> and <i>Scymnus</i> spp.) ..	182	79		
	Mean log (n + 1) Coccinellids per plot	2.22	1.02	±0.31	<2
	Total number of Cecidomyiid larvae ..	33	46		
Total numbers of parasites	<i>Anagyrus pullus</i> Comp.	51	71		
	<i>Clausenia</i> spp.	184	51		
	<i>Leptomastix bifasciatus</i> Comp. ..	1	1		
	<i>Archrysopophagus aegyptiacus</i> Merc. ..	1	2		
	<i>Neodiscodes martinii</i> Comp.	—	21		
	<i>Cheiloneurus carinatus</i> Comp.	—	5		
	<i>Pseudaphycus angelicus</i> (How.) ..	—	10*		
Mealybugs retained for parasite rearing ..		6,283	5,144		

P = probability (expressed as a percentage) that the observed difference between the values for the two habitats could be due to chance.

* Recaptures of an introduced parasite.

Since the effect of using logarithms is to transform an exponential function to a linear one, the correlation coefficient indicates, in reality, the closeness of fit of the field data to an exponential curve, and the regression coefficient represents the geometric rate of change of one factor for a unit change of the other. The correlation coefficients shown in Table III for the three pairs of factors mentioned approximate so closely to unity as to justify fully the use of the logarithmic transformation in handling the field data.

Table III shows that equally high positive correlations between populations and infestation rates of mealybugs were obtained under both cultural conditions. However, when comparing the association of infestation rate and mean number

TABLE III.

Relationships between infestation rates and populations of mealybugs, ants (*Crematogaster* spp.), predators and parasites under cultivated and bush conditions.

	Factors compared	Method of cultivation	Correlation coefficient (r)	P	Regression coefficient (b)
Mealybugs	Number of trees infested and log (n + 1) colonies	Bush Cultivated	+0.98 +0.96	<0.1 <0.1	+1.14 +1.24
	Log (n + 1) colonies and log (n + 1) population	Bush Cultivated	+0.996 +0.995	<0.1 <0.1	+1.84 +1.77
	Number of trees infested and log (n + 1) population	Bush Cultivated	+0.98 +0.98	<0.1 <0.1	+2.11 +2.23
	√Number of trees infested and mean log (n + 1) mealybugs per tree	Bush Cultivated	+0.42 +0.83	non-sig. <0.1	— +0.40
	Number of trees infested and log (n + 1) population	Bush Cultivated	+0.997 +0.865	<0.1 <0.1	+1.32 +1.14
Ants	√Number of trees supporting ants and √number of trees infested with bugs	Bush Cultivated	+0.97 +0.75	<0.1 <0.1	+1.02 +0.81
	Log (n + 1) ant population and log (n + 1) mealybug population	Bush Cultivated	+0.96 +0.75	<0.1 <0.1	+1.52 +1.41
	Log (n + 1) mealybug population and log (n + 1) Coccinellid population	Bush Cultivated	+0.89 +0.65	<0.1 <1	+0.19 +0.04
Predators & Parasites	Log (n + 1) mealybug population and log (n + 1) parasites plus CECIDOMYIDAE	Bush Cultivated	+0.70 +0.80	<1 <0.1	+0.12 +0.17

The first-named factor was taken as the independent variate.
 P = probability of significance of r , expressed as a percentage.

of mealybugs per tree, a significant association was obtained for the cultivated areas but not for the bush sub-plots.

Comparisons were also made of the relationship between (1) the number of trees infested by *Crematogaster* ants and the ant population, (2) the numbers of trees supporting ants and the number of trees supporting bugs, and (3) populations of ants and mealybugs. High positive correlations were again obtained, although the association between the factors was closer in the bush sub-plots than in the cultivated ones.* Similar treatment of the data for predators and parasites showed that the correlations between populations of these and of mealybugs did not differ significantly in the two habitats. It was also shown that populations of *Coccinellid* larvae are larger under bush conditions than in the cultivated areas only when more than 30 per cent. of the trees are infested with bugs. Populations of parasites and *CECIDOMYIDAE* together are equally abundant in the two habitats, irrespective of the number of trees supporting bugs.

Population trends.

Changes in mealybug population may be brought about by changes in the number of infested trees and the number and size of colonies. Analysis of the data showed significant changes between fortnightly sampling periods as regards the number of trees supporting bugs ($P < 5\%$). After grouping the data into four quarterly periods, each consisting of seven fortnightly samples, it was shown that more trees supported bugs during the first three months of the year ($P < 1\%$) than during the subsequent nine, in which the number of infested trees remained constant (fig. 7).

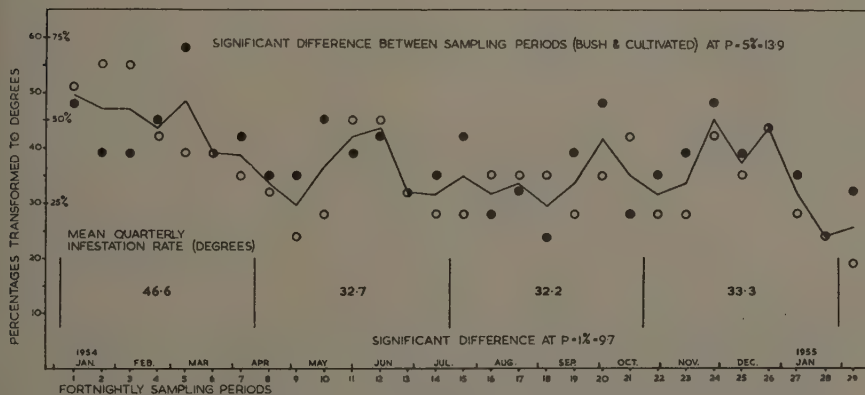


Fig. 7.—Changes in the percentages of trees infested by mealybugs in bush sub-plots (blackened circles) and cultivated sub-plots (open circles).

Because of the close relationship between the mealybug population and the number of trees that support it, seasonal changes in the former become obscured by the marked variations that occur in the latter. To assess population changes on a fortnightly sample of 36 trees, as originally intended, is, therefore, of little value. The population changes described below have been based, instead, on the mean number of mealybugs and colonies of the infested trees only. This treatment of the data has certain limitations: first, not all sub-plots are represented in each fortnightly period, since the trees examined in many of the plots were

* The values of P (expressed as percentages) for the differences between the two correlation coefficients were less than 1, 1 and 5, respectively, for the three comparisons.

not infested; secondly, the assumption is made that changes in population do not produce changes in the number of infested trees and thereby influence the mean population per tree. The writer considers this to be a safe assumption, since it appears that uninfested cacao adjacent to infested trees may remain free from mealybugs for a considerable time, primarily because of the distribution of the mealybug-attending ants. The limitations of the method, moreover, are outweighed by two advantages: first, the marked variations in population caused by changes in infestation rate are eliminated; secondly, the variations being studied are more marked because of the removal of the depressing effect on population

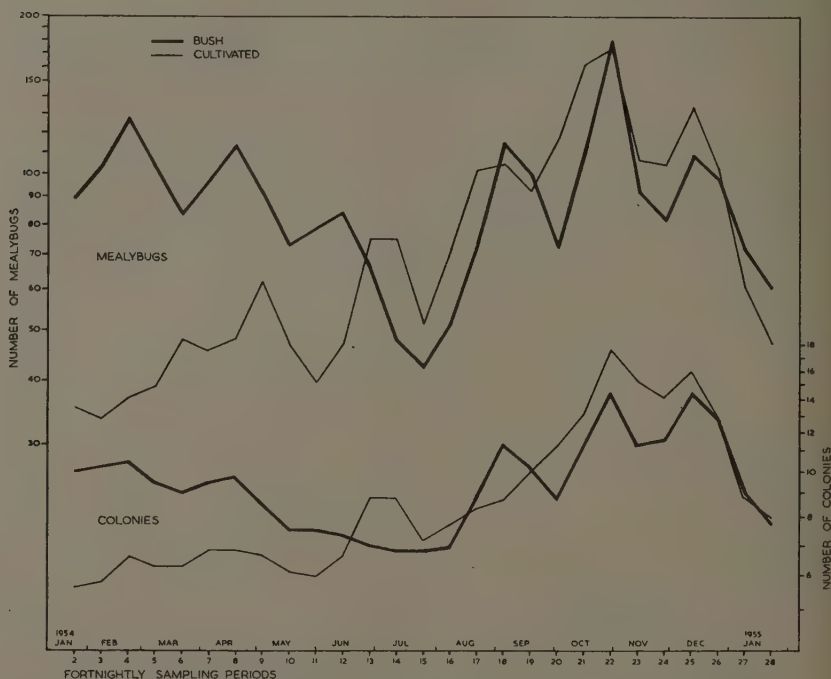


Fig. 8.—Changes in the mean numbers of mealybugs and colonies per infested tree under bush and cultivated conditions. The trends are based on weighted three-position running means.

changes that is due to more than 60 per cent. of the trees having no mealybugs. In presenting the data, weighted three-position running means have been employed; for example, the mean population per tree shown for period two was calculated as $\frac{P_1 + 2P_2 + P_3}{T_1 + 2T_2 + T_3}$ where P and T represent the mealybug population and the number of trees infested, respectively, in any given fortnight. This treatment smooths the variations due to sampling, whilst the weighting of the mean helps to retain the maxima and minima in their correct periods.

Changes in the mean number of bugs and colonies per infested tree are shown in fig. 8. It is evident that changes in mealybug population are determined largely by changes in the number of colonies. Comparison of population trends in the two habitats shows that during the first half of the year the population

rose from 35 to 60 bugs per tree in the cultivated areas and fell from 100 to 40 in the bush areas. During the rest of the year, however, populations in both habitats rose to a maximum of almost 200 bugs per tree. In addition to these major trends, there were marked fluctuations in population in both habitats, giving minor peaks during periods 4, 8, 12, 18, 22 and 26. Adult and nymphal mealybugs showed similar fluctuations (fig. 9).

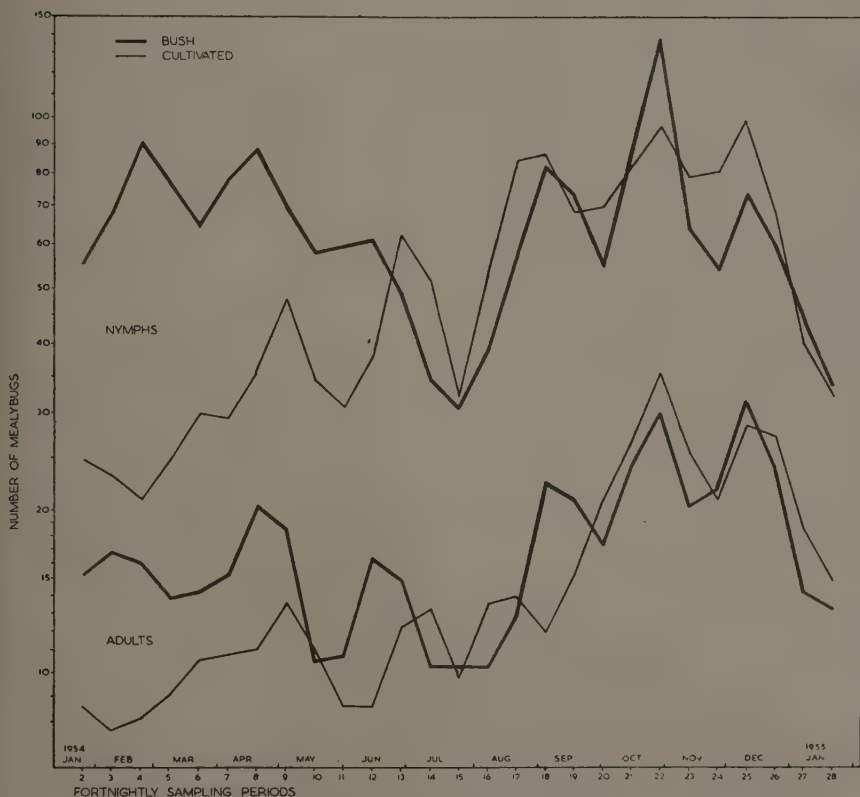


Fig. 9.—Changes in the mean numbers of adult and nymphal mealybugs per infested tree under bush and cultivated conditions. The trends are based on weighted three-position running means.

Changes in population in the two habitats were examined further in five sub-plots having more than half the trees infested, and 13 sub-plots having less than half infested. Fig. 10 shows that the marked drop in population in the bush areas during the first half of the year was caused by changes that occurred in the sub-plots with a high infestation rate; in those having less than half the trees infested, the decline in population was scarcely perceptible. On well-maintained cacao, the rise in population in the first half of the year was attributable to changes in sub-plots where less than half the trees were infested; where more than half were infested there was a slight decrease in the number of bugs per tree (fig. 11).

These results show, therefore, that changes in population are influenced by the degree of infestation. Furthermore, Table IV shows that changes in the number of infested trees do not occur to the same extent in areas of high and low

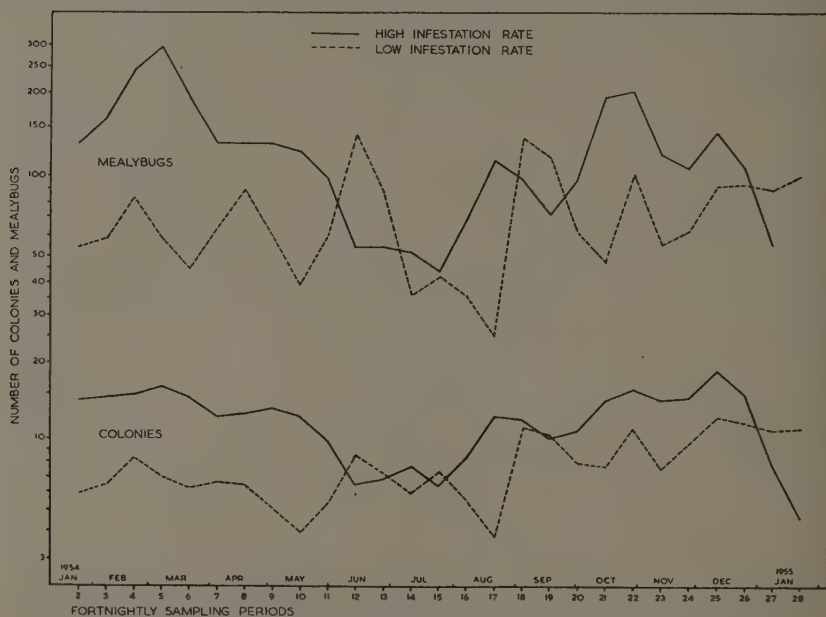


Fig. 10.—Changes in the mean numbers of mealybugs and colonies per infested tree under bush conditions in 5 sub-plots of high infestation rate and in 13 sub-plots of low infestation rate. Trends based on weighted three-position running means.

infestation rate. The data show that in sub-plots with more than half the trees infested, the percentage infestation rate was 9–12 per cent. higher in the first quarterly period than in the second, and where less than half the trees supported bugs, the infestation rate was 12–22 per cent. higher in period I. Differences in the number of infested trees were proportionately greater, however, in the sub-plots where less than half the trees supported bugs, the infestation rates in the cultivated and the bush sub-plots in period I being over 100 per cent. and over 50 per cent. higher, respectively, than those in period II. Comparable figures for the sub-plots having more than half the trees infested were 21 and 12 per cent., respectively. Table IV also shows that in areas of overall low infestation rate, a higher percentage of infested trees support three or less colonies and that changes in the abundance of such trees are more marked in areas of well-maintained cacao. Because of the correlation between vector populations and infestation rates, changes in the former are associated with changes that may occur in the latter. The most reliable indication of seasonal changes in population is provided, therefore, by the trends shown by sub-plots of high infestation rate, in which only slight changes in the number of infested trees occur during the year.

Changes in the mean number of bugs per colony (fig. 12) show four marked maxima in the bush sub-plots and six less-pronounced maxima in the cultivated ones; variations in the percentage of colonies containing 1–3, 4–15 and 16 or more

bugs per colony are shown in fig. 13. The percentage of small colonies (1-3 bugs) fluctuated about 28-30 throughout the year, dropping slightly in November and December. The percentage of colonies of average size (4-15 bugs) increased from the beginning of the year, whilst large colonies were relatively more abundant during the first six months and became fewer towards the end of the year.

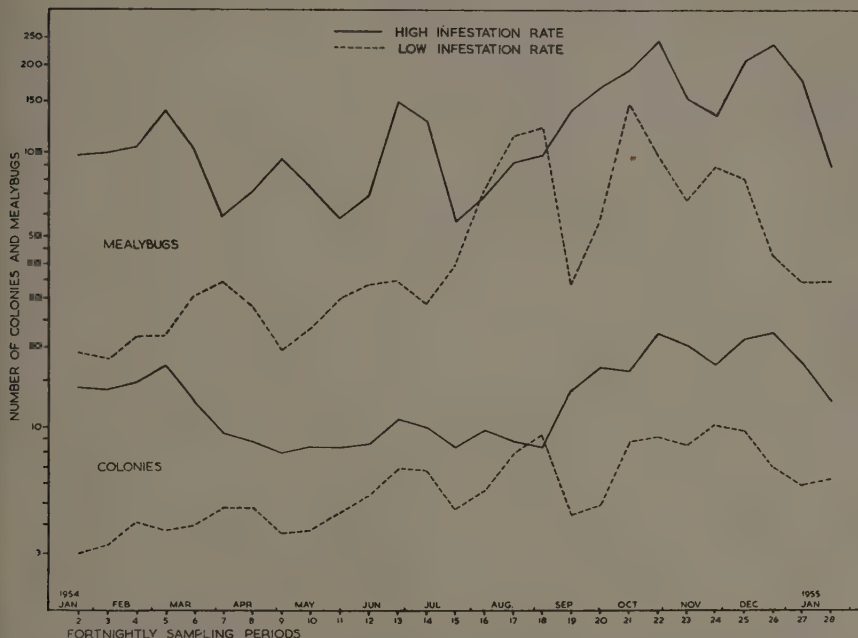


Fig. 11.—Changes in the mean numbers of mealybugs and colonies per infested tree under cultivated conditions in 5 sub-plots of high infestation rate and in 13 sub-plots of low infestation rate. Trends based on weighted three-position running means.

Fluctuations in these trends are not of the same magnitude, and do not necessarily occur at the same time, in the two habitats. Moreover, these fluctuations were found to be unrelated to changes in the mean numbers of colonies or bugs per tree.

An evaluation of seasonal changes in the numbers of ants must be based, on account of their mobile habit, on changes of total population. Fig. 14 shows that more trees were infested with *Crematogaster* ants during the first half of the year. Because of the close association between infestation rates of cacao by bugs and ants, and between the infestation rates and populations of both kinds of insect, changes in the total population of *Crematogaster* ants follow similar trends to those in total mealybug population (fig. 15). Populations of ants of the genus *Pheidole* were too small to allow similar treatment of the data. It is worthy of note, however, that 8 of the 49 trees supporting these ants were recorded during the first sampling period.

Changes in the abundance of predators, shown in fig. 16, are represented by changes in the percentage of mealybug colonies containing Coccinellid or Cecidomyiid larvae. There is an increase in the number of inhabited colonies from the beginning of the year, reaching a maximum between sampling periods

TABLE IV.

Mean percentage infestation rate and percentage of infested trees supporting 3 or less colonies.

	Quarterly periods	Fort-nightly sampling periods	Sub-plots with more than half the trees infested		Sub-plots with less than half the trees infested		Overall mean
			Bush	Cult.	Bush	Cult.	
Mean % of trees infested	I	1—7	83	69	35	43	49
	II	8—15	74	57	23	21	34
	III	16—21	57	74	23	15	32
	IV	22—28	71	60	24	19	34
	Overall mean		71	65	26	24	
Mean % of infested trees supporting 3 or less colonies	I	1—7	3 (0)	17 (8)	34 (13)	54 (28)	
	II	8—15	15 (8)	20 (0)	33 (14)	42 (11)	
	III	16—21	15 (0)	12 (8)	19 (9)	43 (0)	
	IV	22—28	16 (4)	5 (0)	18 (0)	6 (6)	

Figures in brackets indicate the percentage of infested trees having only one colony.

5 and 10, followed by a gradual decrease throughout the rest of the year. Fluctuations in these trends are common to both habitats. The percentage parasitism of adult mealybugs by *Anagyrus* and *Clausenia* (fig. 16) rises to a maximum between periods 4 and 9 and is followed by a decrease throughout the rest of the

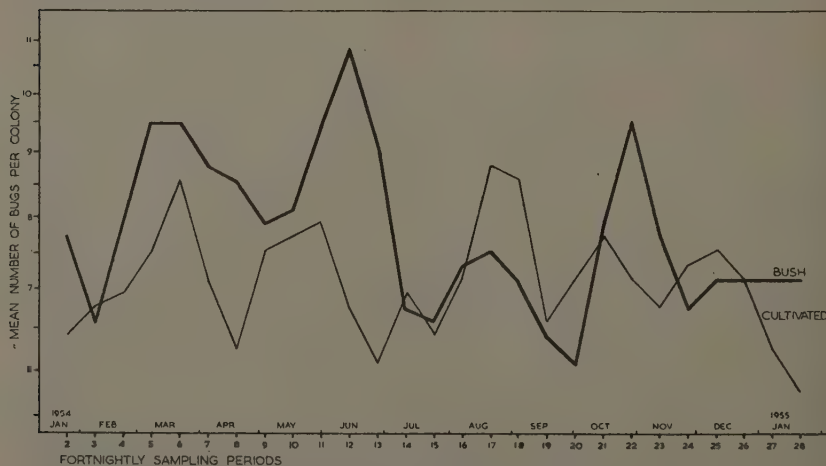


Fig. 12.—Changes in the mean numbers of bugs per colony under bush and cultivated conditions. Trends based on weighted three-position running means.

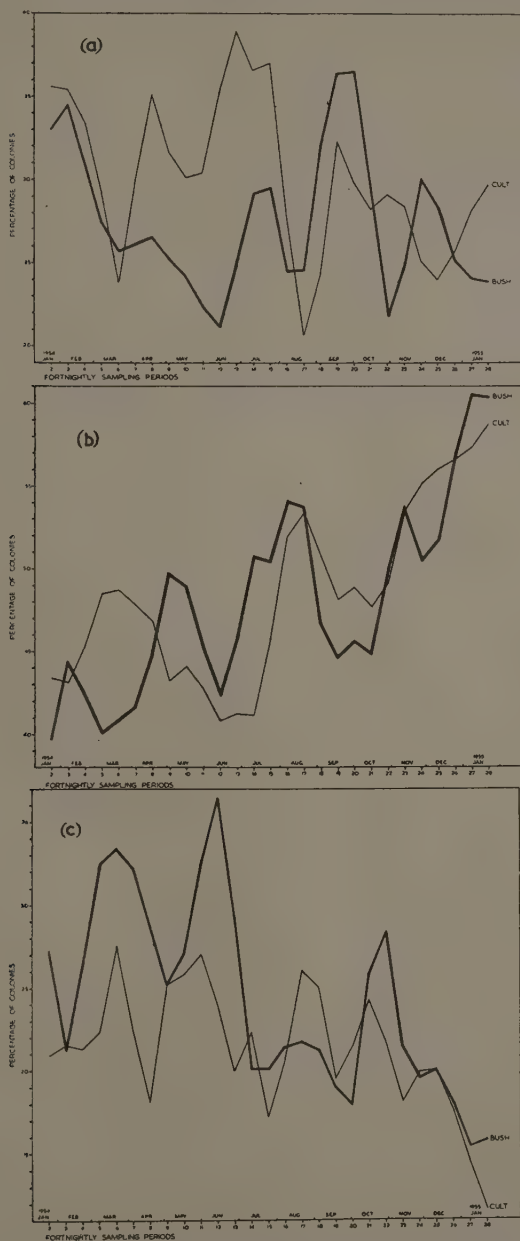


Fig. 13.—Changes in the percentages of colonies containing (a) 1-3 bugs; (b) 4-15 bugs; (c) 16 or more bugs. Trends based on weighted three-position running percentages.

year. This major trend also shows minor fluctuations, which occur at about the same time as those for predators. The initial rise in percentage parasitism and percentage of colonies containing predators (periods 2-5) occurs when the infestation rate of cacao by mealybugs is at a maximum. Parasites and predators are

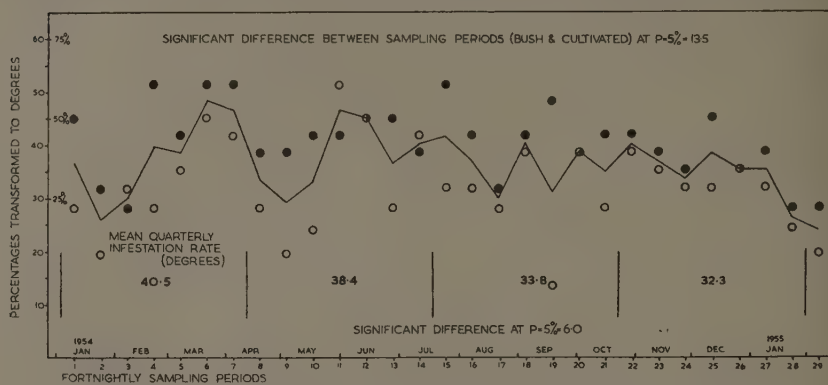


Fig. 14.—Changes in the percentages of trees infested by *Crematogaster* ants in bush sub-plots (blackened circles) and cultivated sub-plots (open circles).

most abundant (periods 5-10) at the time of the decrease in infestation rate between quarterly periods I and II, which is associated with a decrease in the mean number of colonies and bugs per tree. The lowest rate of depredation (periods 21-25) occurs when mealybug populations are at a maximum. Fluctuations in

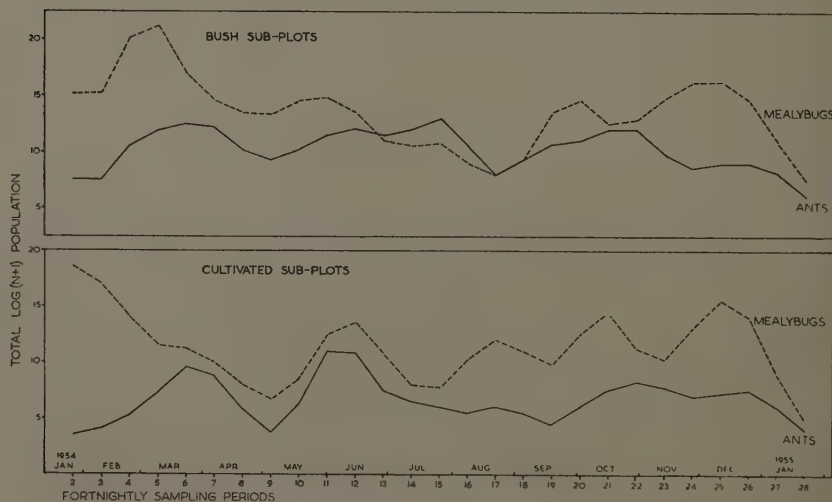


Fig. 15.—Changes in total populations of mealybugs and *Crematogaster* ants under bush and cultivated conditions. Trends based on weighted three-position running totals.

the abundance of parasites and predators are complementary to changes in the mean number of colonies and bugs per tree.

Discussion.

Swollen-shoot disease of cacao is controlled by eradicating, as far as possible, infected material in order to protect healthy trees from infection. There are many problems associated with this principle of "cutting out", the solution of which, while probably not removing the necessity for the continuation of treatment, might at least make the cutting-out campaign more efficient and economical. Measures directed against the vectors of virus diseases are unlikely to provide

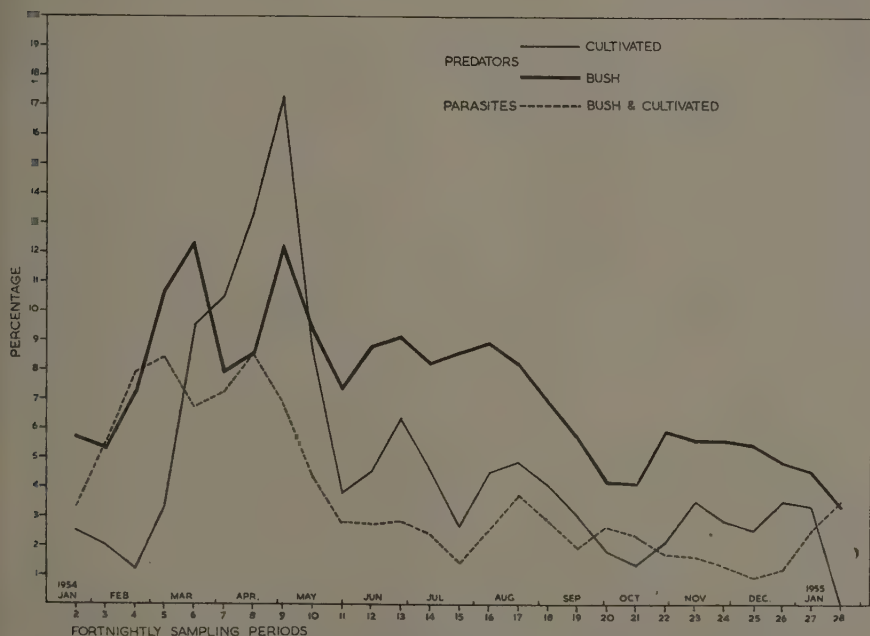


Fig. 16.—Changes in percentage parasitism and in the percentage of colonies containing predators. Trends based on three-position running percentages.

successful methods of disease control; nevertheless, if there were no vectors there could be no transmission of the disease. The mealybug vectors of cacao viruses occur at a mean density of only seventy insects per tree (Strickland, 1951a), and it appears to be a matter of extreme difficulty to reduce their numbers still further.* Mealybugs, however, are often extremely localised in distribution

* The introduction of exotic parasites and predators has been attempted, but the method is unpromising and only one such species shows signs of establishment. Insecticidal sprays and dusts are of little value, partly because of the cryptic habits of the vectors and the protection afforded to them by tent-building ants, and partly because of the practical difficulties in applying such insecticides under the conditions encountered on the average farm in Ghana. Systemic insecticides have, on an experimental scale, produced a higher mortality of mealybugs than any other agent yet tried, but the cost of using them on a large scale appears to be prohibitive and there is still no evidence that their use has been able to reduce the rate of dissemination of swollen shoot disease. In addition, the practical application of systemic insecticides is limited by

and there are large areas of cacao in which not a single vector can be found. This suggests that it might be possible to vary the environmental conditions so as to make them utterly unsuitable for mealybug survival.

The two habitats examined in this survey were selected for study because they are representative of extreme types of cacao cultivation found in Ghana. Whilst few farmers maintain their cacao at the high level of crop sanitation described for the cultivated sub-plots, the majority of farms are found in increasing stages of deterioration, many apparently abandoned and typified by the conditions described for the bush sub-plots. The survey has shown that the environment of the two habitats does not significantly influence the number of cacao trees infested by mealybugs or the size of the vector populations. There is, accordingly, no reason to believe that artificial adjustment of the vegetation associated with cacao in the field would provide a method of reducing mealybug populations to the very low level necessary for a reduction in the rate of virus incidence. On the contrary, infestation rates varying from about one to twenty-four trees per sub-plot were recorded in either habitat. Nevertheless, the effects on the vectors of the type and density of overhead shade and of the spacing of the crop remain to be studied further.

The survey has demonstrated extremely close correlations between populations and infestation rates of the sub-plots by vectors and *Crematogaster* ants, particularly where cacao is growing in bush. This result confirms the observations of Strickland (1951b) and emphasises the need for further study of the ant-mealybug association. The close association between the number of trees infested by mealybugs and the mean number of mealybugs per tree which is observed in well-maintained areas, but not in the bush, may be attributed to the comparative absence of host-plants other than cacao in the cultivated areas, in contrast to the abundance of such plants amongst the bush vegetation where cacao is poorly maintained.

The survey has shown that more trees were infested with bugs during the first three months of the year and that changes in infestation rate were most marked in areas where few trees were infested. It was thought that the first of these results might have been induced by the physical effects of sampling. Examination of Strickland's data for seven plots in which less than 40 per cent. of the trees were infested (Strickland, 1951b) shows a significant increase in the number of trees infested during 1947-48, the figures for successive two-monthly periods from May-June 1947 to March-April 1948 being 29, 39, 35, 52, 61 and 63. Since Strickland's survey was started in May, it seems unlikely that the higher infestation rate during the early months of the year in the present survey was brought about by the sampling technique. Strickland (1950) showed that wind dispersal of the vectors is most marked during the dry season. Furthermore, experience has shown that a large number of trees first show symptoms of infection by swollen-shoot virus during April and May. This has been attributed to the flushing of cacao at this time of the year facilitating the task of detecting symptoms. Nevertheless, three pieces of circumstantial evidence suggest that virus spread may occur more readily during the dry season.

Strickland (1951b) could find no consistent differences in vector population between monthly sampling periods. His analyses were based on total populations, which are influenced by changes in infestation rate. The present work has shown that a better indication of changes in population is obtained from a study of the mean population per infested tree. Changes in this value are influenced by the extent of infestation of the area under study. Using this method, however, it is evident that there are consistent changes in population in scattered plots and that vector populations may increase five or six-fold during the course of a few

their high toxicity to man and the tainting of cacao beans which make them unacceptable to the manufacturers.

months. Data for 1947-48 show an increase in the geometric mean of the number of mealybugs per tree from 47.2 during the first six months of the year to 71.8 during the last six months. These figures may be compared with an increase in the overall geometric mean (bush and cultivated areas) of 64.9 during the first six months to 91.7 during the latter half of 1954. These figures represent increases in the mean of 66 and 70 per cent. in the two surveys.

Changes in the size of mealybug colonies appear to be of minor importance in determining changes in total population. Factors which are of importance are those that influence the formation and destruction of whole colonies. From the data available, it appears that the reduction in infestation rate and the decrease in the mean number of colonies per tree between quarterly periods I and II in the present survey were brought about by parasitism and predation by indigenous natural enemies. From figs. 8 and 16 it appears that changes in population are brought about by a balance of host, predator and parasite populations.

Examination of the meteorological data shows that the decline in vector population during the first half of the year and the rise during the second half were both associated with periods of increasing rainfall. Changes in maximum temperature and in saturation deficit show trends similar to those of the vector population, but the marked fluctuations in the latter are not associated with similar changes in atmospheric factors. The effect of climatic factors on mealybug reproduction and length of life-cycle are unknown. Strickland (1951b) considers that there may be eight generations per annum. It is possible that the marked fluctuations in the abundance of mealybug colonies during the year may be brought about by six reproductive cycles. This is considered unlikely, however, since there is no evidence to suggest that reproductive cycles should be in phase in all plots or even on all trees within the same plot. Furthermore, the trends shown for populations of adults and nymphs indicate a steady balance of mature and immature stages throughout the year.

Summary.

A comparison is made of the populations of mealybugs, ants, predators and parasites on cacao growing in dense secondary bush and under well-cultivated conditions in Ghana, West Africa. The percentage of cacao trees infested (termed the infestation rate), the number of colonies and the populations of the vectors were similar under the two cultural conditions. Populations of ants of the genus *Crematogaster* and of COCCINELLIDAE were higher in bush areas than in cultivated ones. Populations of ants of the genus *Pheidole*, though smaller than those of *Crematogaster*, were greater in areas of well-maintained cacao.

Correlations of infestation rates and populations were compared under the two cultural conditions for mealybugs, ants, predators and parasites. The field data showed an exponential relationship between these factors, which was rendered linear by suitable transformations. The correlation of mealybug populations with infestation rate and with populations of parasites and predators was equally high in both habitats. The correlation of mealybug populations with those of *Crematogaster* was closer in the bush than in well-maintained cacao, but the implications of this are not fully understood.

There was a higher infestation rate of cacao during the first three months of the year. In contrast with previous work, the present survey has demonstrated significant changes in the vector population during the year, of a five- or six-fold order. These seasonal changes are based on mean mealybug populations per infested tree, and were largely the result of changes in the numbers of colonies; populations showed a decline during the first six months of the year and then rose to a maximum during October-November. The data suggest that these trends may be brought about largely by changes in the abundance of predators and parasites.

Acknowledgements.

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TRIALS ON THE UNDERGROUND STORAGE OF MAIZE OF HIGH MOISTURE CONTENT IN TANGANYIKA.

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(PLATE IX.)

The storage of food grains in underground pits is a well-established practice in a number of South American countries such as Argentina (which, according to Hall & Hyde (1954), has storage for about 2,000,000 tons), Paraguay, Venezuela and Uruguay. Preliminary trials in Tanganyika (Swaine, 1954) indicated that the method was satisfactory for the long-term storage of maize in the Territory, and the construction of pit storage to hold grain as a famine reserve was begun in 1953. In this method of storage, dry grain, that is, grain of a moisture content less than 13.5 per cent., is sealed down in bulk in an airtight pit in which the respiration of the insects present in the grain brings about an accumulation of carbon dioxide and a depletion of the oxygen from the intergranular air. Under these conditions the insects are killed and any further damage to the grain prevented. Although it has now been established by S. W. Bailey, working at Canberra, Australia, that deficiency of oxygen is the factor responsible for killing insects placed in hermetically sealed containers (Department of Scientific & Industrial Research, 1956, p. 20), it was previously thought that increase in carbon dioxide was the responsible factor and for that reason changes in the concentration of this latter gas only were measured in the trials reported here.

The decision to explore the possibilities of the underground storage of maize of high moisture content in Tanganyika arose from a suggestion put forward by the Pest Infestation Laboratory, Department of Scientific and Industrial Research, Slough, following on recent experience in France on the hermetic storage of food grains of high moisture content in above-ground silos (Hall & Hyde, 1954).

The costs of the trials reported here have been equally shared by the Governments of Uganda, Tanganyika and Kenya. The maize was supplied by the Uganda Government and the trials were carried out in the 120-ton Tanganyika pits situated at Moshi and Morogoro. These two pits were the ones used in previous trials (Swaine, 1954), the main differences being that the former water-vapour/gas barrier facing of two coats of bitumastic paint, sand and a final coat of white oil paint had been removed from the walls and replaced by one consisting of a rendering of cement (3 cement: 1 sand) $\frac{1}{2}$ -inch thick, two coats of red ruberine paint and one coat of white oil-bound paint. The sealing layers on the Moshi pit consisted of two layers of "Ruberoid" roofing material with a separating layer of chicken wire, the whole being covered with hot bitumen and finally painted with aluminium paint: sealing of the Morogoro pit consisted of an underlayer of asphalt saturated felt (concreting paper) and an overlayer of Pluvex no. 1 hessian base damp course. The two sealing layers were covered, as soon as laid, with hot bitumen. No intermediate layer of chicken wire was used and no final coat of aluminium paint was applied.

Sampling Methods.

Grain samples were removed by a grain spear inserted into the bulk through holes cut in the roofing material. Grain temperatures were recorded by a slow-moving thermometer, suitably mounted on a wooden spear, and inserted as

required through the roofing material. Later temperatures for the Morogoro pit were obtained by suspending slow-moving thermometers at known depths in 1½-inch iron piping buried in the bulk.

Intergranular air samples were taken with the apparatus devised by Pritchard & Walton (1952), dirt and dust being kept out of the suction pump by interposing a trap bottle, containing cotton-wool, between the pump and the ½-inch gas piping through which the samples were drawn from the pit (Pl. IX, fig. 1). Atmospheric air in the apparatus was first removed by 10–40 double strokes of the pump, depending on the depth of the required sample, 0–11 ft.; the metal sample cylinder, discharged from previous sampling, was then attached and filled with a further 10 double strokes of the pump. Carbon dioxide concentrations were determined by the Oxley CO₂ apparatus (Oxley, 1944) after equilibration of the gas temperature of the samples with that of the laboratory by standing overnight. As the Oxley apparatus in use was incapable of recording CO₂ concentrations higher than 20 per cent., the pit samples were diluted, where necessary, with known volumes of air in a graduated hypodermic syringe prior to determination. Fine regulation of the gas flow from the sample cylinder was achieved by the insertion of a light spring under the head of the reducing valve supplied with the Pritchard & Walton gas sampling apparatus such that a very slow and controlled rate of flow could be obtained by pressure of the hand.

Determinations of moisture content were made either by a standard oven-drying method or by calibrated Marconi meter. Germination tests were made with seeds outwardly normal in appearance and not damaged by insects. Palatability tests were carried out by Africans, whose comments were scored on a pre-arranged scheme.

Moshi Pit Experiment.

The maize on arrival at the Moshi depot from Uganda was in a generally dirty condition; it was estimated that approximately one-third showed signs of mould in varying degree, whilst some bags were so wet that the grains had begun to germinate. The grain from these very wet bags was not used in filling the pit. The fungi were identified by Dr. G. B. Wallace, formerly Plant Pathologist, Agriculture Department, Tanganyika, as *Aspergillus* spp., *Nigrospora sphaerica*, *Fusarium graminearum* and *Penicillium* spp. Unidentified yeast fungi were later isolated by Dr. Wallace in culture.

Moisture content.

The moisture content of the maize was extremely variable, showing readings on the Marconi meter from 13.5 to 20.0 per cent. The average of 39 readings on the first two consignments, which arrived on the 19th and 20th September 1954, was 15.6 per cent.; that of 61 readings on the last consignment, which arrived on 24th September 1954, was 17.7 per cent.

Infestation.

The insect infestation, consisting of *Calandra oryzae* (L.), *Oryzaephilus surinamensis* (L.) and *Tribolium castaneum* (Hbst.), was generally very light, although very large numbers of the two latter species were present in odd bags. In sampling, counts were made of the numbers of *C. oryzae* only.

Post-sealing changes.

Results of regular tests made after sealing are given in Table I. Nineteen days after sealing, an unpleasant beery smell was observed in gas samples removed from the pit. The grain itself appeared unchanged up to 83 days after sealing, but a deterioration in colour was noted in a sample taken 118 days after sealing, the grains in this sample having become slightly brownish-yellow, whilst

TABLE I.

Sampling data for the Moshi pit.

Time of sampling	No. of <i>C. oryzae</i> per lb. grain		No. of <i>C. oryzae</i> emerging per lb. grain up to 30 days after sample was taken	Damaged grains (%)	Grain temperature at depth below 3 ft. (°C.)	CO ₂ in inter-granular air (%)	Moisture content of grain (%)	Germination of grain (%)	Grain colour
	Alive	Dead							
Before sealing	3.5	0.0	5.2	1.4	—	—	16.9	83.0	Creamy white
Days after sealing									
0	—	—	—	—	—	2.94	—	—	“
1	—	—	—	—	29.6	8.37	—	—	“
2	—	—	—	—	—	11.82	—	—	“
4	1.0	0.0	—	—	28.5	11.82	—	—	“
19	—	—	—	—	27.6	18.41	—	—	“
34	—	—	—	—	29.0	22.72	17.1	92.0	“
47	—	—	—	—	30.9	25.63	—	88.5	“
83	—	—	—	—	31.3	31.75	—	63.3	“
118	0.0	0.4	—	0.3	*35.0	35.42	—	6.0	Brownish-yellow

Pit sealed 25.ix.54.

* One sample only.

the top few inches of grain showed considerable darkening. Storage up to 118 days was accompanied by a steady increase in CO_2 concentration up to 35 per cent. and a decrease in grain viability from an initial 80-90 per cent. to 6.0 per cent. (Table I).

On the basis of the above changes, a palatability test was carried out on a sample taken 129 days after sealing in comparison with samples from maize provided for the purpose by the Department of Grain Storage and the Department of Agriculture, Morogoro. The results (Table II), whilst not conclusive, did indicate that the pit maize was only fairly satisfactory as food. A distinct sour smell could be detected in the meal 24 hours after grinding, but this had become appreciably less sour after a further 24 hours.

TABLE II.

Palatability tests on Moshi pit maize compared with two Morogoro samples.

Taster	Origin of maize sample		
	*Moshi pit	Morogoro D.G.S. store	Morogoro Agriculture Department
1	Very good	Bitter	Very good
2	Very bad	Slightly bitter	Good
3	Very bad (smells)	Very good	Very good
4	Good	Good	Very good
5	Good	Good	Good
6	Bad	Bad	Very good
7	Fairly good (smells)	Very good	Very good

* Pit sample taken after 129 days' storage.

The results of the palatability tests, together with the recorded high concentration of CO_2 and loss of viability of the seed, led to a decision to open the pit to see what gross changes had taken place and to decide how best to dispose of the maize.

Observations on the opening of the Moshi pit.

The pit was opened on 16th February 1955, 144 days after sealing, in the presence of Government and commercial observers from Uganda, Tanganyika and Kenya.

A strong beery smell accompanied the removal of the sealing layers. General grain appearance was voted reasonable compared with normal Uganda maize. Heavy caking, 2-3 ft. in thickness in some places, was present on the side and end walls. The caked grain was covered with a conspicuous white fungal growth, provisionally identified by Dr. Wallace as a *Monilia* sp. Other fungi present were *Aspergillus* sp., *Cladosporium herbarum* and an unidentified Actinomycete. No caking was observed on the bottom of the pit where the grain appeared in very good condition. The caked maize, amounting to approximately 1,400 lb., was evidently unfit for human consumption but it was considered that the remaining bulk of the maize might be acceptable after conditioning, or conditioning and admixture with other grains. Tests carried out by the Provincial Produce Office, Department of Grain Storage, Moshi, indicated that admixture at

TABLE III.
Sampling data for the Morogoro pit.

Time of sampling	No. of <i>C. oryzae</i> per lb. grain		No. of <i>C. oryzae</i> emerging per lb. grain up to 30 days after sample was taken	Damaged grains (%)	Grain temperature at depth below 3ft. (°C.)	CO ₂ in inter-granular air (%)	Moisture content of grain (%)	Germination of grain (%)	Remarks
	Alive	Dead							
Before sealing	2.29	0.62	9.1	4.3	—	—	14.8	64.3	
Days after sealing									
0	—	—	—	—	30.0	9.96	—	—	
1	—	—	—	—	—	12.33	—	—	
3	0.0	3.43	7.2	—	—	12.84	—	—	
4	—	—	—	—	—	13.52	—	—	
8	0.0	—	—	—	—	13.97	—	—	
29	0.0	—	—	—	—	17.23	—	—	
49	0.0	—	—	—	—	16.62	—	—	
53	0.0	1.6	0.0	—	—	17.64	—	*0.0	Beery smell in air sample
71	0.0	3.7	0.0	3.6	—	19.63	14.5	21.3	Strong beery smell
83	0.0	9.0	0.0	3.6	31.0	21.30	14.9	—	*Sample from top only
88	—	—	0.0	—	—	—	—	13.3	Bottom samples most viable
138	0.0	2.8	0.0	—	30.8	19.50	—	1.5	Only bottom samples viable
179	—	—	—	—	30.8	—	—	—	
243	0.0	4.4	0.0	—	30.0	17.70	—	0.0	
281	—	—	—	—	30.0	19.40	—	0.0	

Pit filled 2.xii.54 and sealed 3.xii.54.

the rate of one part Uganda maize from the pit to two parts of maize from the Moshi store, followed by conditioning, would produce an acceptable meal. The admixed maize was subsequently offered to the millers and the public, no complaints of any kind being received.

Morogoro Pit Experiment.

The main differences from the Moshi experiment were that the grain was in a much cleaner condition, of lower average moisture content, 14.8 per cent. compared with 16.9 per cent., and that only a very few samples showed mould at the bases of the grains.

Moisture content.

The average of 30 samples tested with the Marconi meter was 15.0 per cent., that of 10 separate samples determined by standard oven drying was 14.3 per cent., giving an overall average from the 40 samples of 14.8 per cent. The moisture content was more uniform than that of the grain for the Moshi pit, ranging only from 13.8 per cent. to 16.4 per cent. in the samples tested.

Infestation.

Insect infestation was fairly light and uniform throughout the whole consignment. The total number of live insects sieved from 15 × 14-oz. samples was: *Calandra oryzae*—101, *Tribolium castaneum*—17, *Oryzaephilus surinamensis*—5.

Post-sealing changes.

Whilst the precise number of days required to eliminate the insect infestation was not recorded, it must be concluded that death was fairly rapid. No live insects of any species were observed in samples taken 29 days after sealing and none emerged from grains taken 53 days after sealing. The insect records in Table III are concerned solely with *C. oryzae*, as this is the most destructive grain pest in Tanganyika. Samples taken from the pit were first sieved to record the numbers of *C. oryzae* (Table III, col. 2) and then kept for 30 days to record the numbers that emerged (Table III, col. 3). The same beery smell such as was noticed in the Moshi pit was present 29 days after sealing and persisted throughout the storage period. Grain in the top six inches had become dark brown in colour and was dead, 53 days after sealing. Viability of the bulk of the maize decreased, from an average of 64.3 per cent. at the beginning of storage, to nil, 243 days after sealing (Table III). Sampling up against the walls of the pit failed to reveal any caked or heavily moulded grains up to 281 days after sealing.

Palatability tests, carried out at three different intervals after sealing, on mixed samples obtained from a depth below 3 ft., are reported on in Table IV. In these tests all grains, except those from which the bought samples of meal had been prepared, were ground by hand mill and then sieved through a 0.5-mm. sieve before cooking. Each sample was tested by 17 individuals whose comments were scored: very good (3), good (2), good but smells (1), good but slightly bitter (0), slightly bitter (-1), bitter and smells (-2), bad (-3), very bad (-4). The Morogoro pit maize meal was dark in colour; 258 days after sealing it had a pronounced sour smell and bitter taste which could not be removed even after airing for 13 days and it was considered that some admixture with a good grain would be required to making it acceptable to the public. Test D, Table IV, indicates that a 1:1 admixture would have been acceptable, the resulting score being considerably better than that of maize meal bought from the shops in Morogoro.

TABLE IV.
Palatability tests on maize from the Morogoro pit and other sources.

Test	Source of sample	Score awarded	Days after sealing	Remarks
A	1. Morogoro pit	0	83	
	2. Morogoro farm	+33		
	3. D.G.S. store (1954 maize)	+17		
	4. Bought maize meal	+1		
B	1. Morogoro pit	-33	258	
	2. Morogoro farm	+48		
	3. D.G.S. store (1955 maize)	+9		
	4. Mwanza maize	+11		
C	1. Morogoro pit	-31	272	Maize from Test B, retested after airing for 13 days
	2. Morogoro farm	+25		
	3. D.G.S. store (1955 maize)	+21		
	4. Mwanza maize	+40		
D	1. Morogoro pit	-31	285	Cooked meal brown Cooked meal slightly brown Cooked meal very slightly brown Cooked meal creamy white Cooked meal creamy white Cooked meal grey
	2. Morogoro pit—Morogoro store 1 : 1	+15		
	3. Morogoro pit—Morogoro store 1 : 2	+22		
	4. Morogoro pit—Morogoro store 1 : 3	+17		
	5. Morogoro store	+43		
	6. Bought maize meal	+3		

Observations on opening the Morogoro pit.

The pit was opened on 12th December 1955, 347 days after sealing, observers including representatives from Uganda and Kenya and Mr. T. A. Oxley, Pest Infestation Laboratory, Slough. As in the Moshi pit, considerable darkening of the grains in the surface layers was immediately apparent. Caked and mouldy grains were present, mostly on the inward sloping side walls (Pl. IX, fig. 2), but the degree of caking was by no means as heavy as that observed on opening up the Moshi pit. A curious feature of this caking was that it began at a depth of about six inches rather than at the lip of the pit. Maize at the bottom of the pit was not caked.

No ready sale at a reasonable price could be obtained in Morogoro and the maize was railed back to Uganda for conditioning and suitable admixture with a more palatable grain before being put on the market.

Discussion.

As in previous experiments, with grain lower in moisture content, the insect population died out and no increase in insect damage to the grain was recorded. However, as the storage life of the better of the two parcels under test was only a little over one year and the maize on removal was not palatable without admixture with a good grain, it is clear that the hermetic storage of maize of high moisture content under tropical conditions is not a practicable proposition. This is in general agreement with C. O. Lopez (Argentina, Ministerio de Agricultura, 1949), who worked with wheat, of moisture contents from 12.5 to 17.5 per cent., sealed in airtight bottles. He concluded that grain destined for underground storage should be of less than 13.5 per cent. moisture content and that the internal grain temperature should not rise above 25°C. The success of the French experiments in the above-ground hermetic storage of wheat of high moisture content would appear to be due to the lower atmospheric, and hence grain, temperatures.

Death and browning of the grains in the surface layers is undoubtedly connected with the excessive heating due to the sun. Some indication of the temperature which can be reached in the upper layers of grain and the large diurnal variation which is experienced under tropical conditions is given in Table V.

It is of some interest that, even when a protective coating of aluminium paint was applied to the covering layers (Moshi pit), death and browning of the surface grains still occurred.

The reason for the caking occurring only on the walls, and not the floor, of the two pits is not clear. The differences in grain and soil temperatures on either side of the pit walls given in Table VI do not seem big enough to have caused condensation of water at the concrete face of the pit wall. A temperature of 38.8°C. recorded for the caked maize at a depth of 7 in. compared with 37.6°C. for maize at the same depth, but in the centre of the pit, indicated that no undue heating developed in the grains near the wall. Differences were recorded in moisture content: 12.6-13.7 per cent. in the top 6 in. or so of maize beneath the sealing layers, 14.8-16.4 per cent. in the bulk of the maize below 3 ft., and 16.7-21.7 per cent. in caked maize. It is possible that the excessive heating of the grain in the surface layers, due to insolation, resulted in a movement of water vapour therefrom and the development of high intergranular humidities at the walls at a point just below that subject to the influence of the sun's rays, and from which caking spread.

Summary.

Trials were carried out, at two places in Tanganyika, on the underground storage in sealed pits of maize of high moisture content. The average moisture

TABLE V.

Variations in temperatures—Morogoro pit.

Date	Time	Grain temperature (°C.)			Temperature on outside rubberoid cover (°C.)	Ambient air temperature (°C.)
		Depth		11 ft.		
		1½ in.	3 in.			
27.iv.55	1400 hr.	38.9	36.6	29.4	40.0	28.0
28.iv.55	0930 "	31.2	30.0	29.5	40.0	25.0
28.iv.55	1400 "	50.0	42.2	29.5	55.6	29.4

TABLE VI.

Comparison of grain and soil temperatures—Morogoro pit.

Date	Days after sealing	Temperature (°C.)		
		Grain, against side wall	Ground, against side wall	
		Depth, 5 ft.	Depth, 6 ft.	Depth, 10 ft.
2.xii.54	0	30.0	—	—
1.iii.55	83	31.0	—	—
27.iv.55	138	30.8	—	—
5.v.55	146	30.5	29.2	29.0
30.v.55	179	30.8	28.9	29.0
2.viii.55	243	30.0	27.0	28.0
9.ix.55	281	30.0	27.2	27.8

content of the maize when placed in the two pits was 16.9 and 14.8 per cent., whilst the general level of insect infestation was very light and fairly light, respectively. The predominant species was *Calandra oryzae* (L.); *Oryzaephilus surinamensis* (L.) and *Tribolium castaneum* (Hbst.) were also present.

The maize was kept in the sealed pits for 144 and 374 days, respectively. Samples taken at intervals showed that the insect infestation died out early, and there was no increase in insect damage. Other changes, unconnected with insect infestation, took place, and conditioning and admixture of a more palatable maize was necessary before grain from the pits could be offered to the public.

It is concluded that long-term underground storage of maize of high moisture content is not a practical proposition under tropical conditions owing to the deterioration that occurs in the grain.

Acknowledgements.

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FIG. 1. Apparatus for the withdrawal of air samples from pit. Sample cylinder attached at right angles to pump.



FIG. 2. Maize caked on the wall of the Morogoro pit. Caking ends at some short distance from top of the pit.

A STUDY OF THE BLACKFLY, *SIMULIUM ORNATUM* MG. (DIPTERA), WITH PARTICULAR REFERENCE TO ITS ACTIVITY ON GRAZING CATTLE.

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The present work was carried out to provide quantitative information on the activity of the blackfly, *Simulium ornatum* Mg. (Diptera, SIMULIIDAE), on cattle under various weather conditions, and at different times of day. A useful basis for the work was afforded by Smart (1934) who elucidated the main features of the annual cycle in this species, and Zahar (1951) provided additional biological information and made preliminary studies on the activity of females on cattle in Scotland. The rôle of *S. ornatum* as vector of the nematode, *Onchocerca gutturosa* Neumann, parasitic in cattle, demonstrated by Steward (1937), formed a further incentive to study the fly in more detail.

The work was carried out during 1952-54 in a lowland area 5 km. east of Durham, England (lat. 54°46'N.) where cattle pastures are situated adjacent to a small stream, averaging 1 metre in width, in which the larvae and pupae of *S. ornatum* occurred at great density (Nat. grid ref. 45/3341). In 1952 only, ancillary observations were made on the seasonal changes in the numbers of the aquatic stages and on oviposition activity.

The results of a study of the age composition of *S. ornatum* fly-catches obtained off cattle will be reported in a later paper. A preliminary account has already been published (L. Davies, 1955).

Seasonal Occurrence of the Aquatic Stages.

In batches of 200-400 pupae taken from the stream on many occasions, *S. ornatum* was frequently the only species present. Occasionally *S. (Eusimulium) latipes* (Mg.) and *S. (E.) aurum* Fries were present but formed less than 1 per cent. of the total. In view of the preponderance of *S. ornatum* in this stream, samples taken regularly for counting were assumed to consist of *S. ornatum* only.

At weekly or fortnightly intervals in 1952, five small stones of around 5 × 5 × 3 cm. in size and typical of the substratum of the rapid portions of the stream, were removed from each of two sample stations, each station being a rapid about two metres long. The stones were placed in a metal can containing formaldehyde, and all larvae and pupae removed in the laboratory by hand and by flotation in magnesium sulphate solution. Since the distribution of the aquatic stages of *Simulium* was highly discontinuous even within a distance of two metres, the figures obtained by the above sampling method are no doubt influenced by large sampling errors so that some fluctuations in the numbers of larvae and pupae in samples occurred without relation to real changes in total abundance in the particular part of the stream. The effects of this fluctuation were reduced by calculating the mean of the figures for the two stations and expressing them as the log of the number per stone, plotted in fig. 1. Larvae were classified by eye into small (less than approx. 4 mm. length) and large (over 4 mm. length), and the curve for pupae includes pupal exuviae as well as unhatched pupae. The latter procedure was adopted in view of the relatively short pupal period of 4-8 days at summer temperatures (Smart, 1934) as compared with larval duration (probably at least 14 days) and the intervals between sampling. Moreover,

periodic examination of marked stones showed that pupal exuviae usually disappeared within a week in this type of stream.

A serious source of inaccuracy in the sampling, as an index of fly production from the stream, lay in the fact that, from June to October only, considerable numbers of larvae and pupae occurred on vegetation trailing in the stream, a substratum which it was not possible to sample reliably.

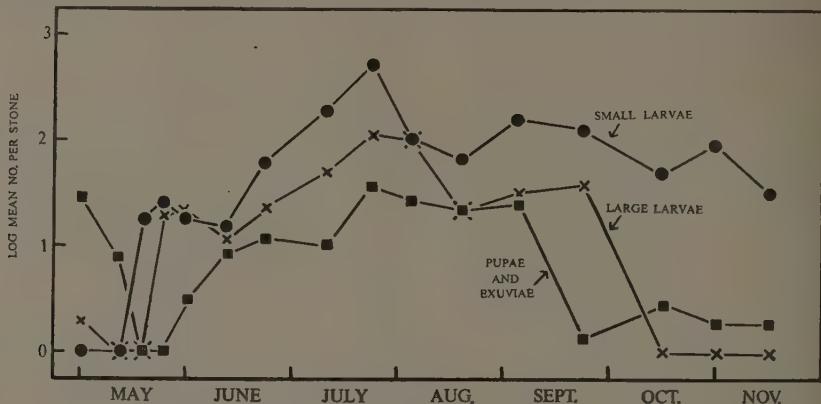


Fig. 1.—Variation in abundance of the aquatic stages of *S. ornatum*, 1952 season.

S. ornatum overwinters in the larval stage (Smart, 1934) and sampling began in May, when emergence of spring adults from overwintering larvae was ending. Small larvae, the progeny of spring adults, did not appear in samples until late May (fig. 1) and remained at a concentration of over 10 per stone for the remainder of the season, rising to a maximum of over 500 per stone in late July. The number of pupae and exuviae remained at 10–40 per stone from early June to late September. The much lower numbers of pupae and exuviae than of larvae of either category in samples was presumably a reflection of heavy larval mortality.

The curve for pupae indicates continuous breeding from late May onwards rather than the occurrence of separate generations, while in the case of large and small larvae it is considered that the smaller peak in late May and the succeeding larger peak in late July may represent successive generations. The numbers of pupae and exuviae on stones had dropped to a low level by late September (fig. 1), a fact confirmed by examination of many stones from other parts of the stream. Large numbers of pupae were, however, still present on trailing vegetation throughout September and up to at least 25th October 1952. The occurrence of pupae on vegetation much later than on stones cannot be explained. Irregular observations in the autumn of 1953 produced a similar picture. The occurrence of pupae of *S. ornatum* in numbers as late as mid-October confirms the remarks of Edwards (1920), and the findings of Smart (1934) working in south-eastern Scotland. These results suggest that following mass emergence of flies, derived from overwintered larvae, during April and early May, there should be a relatively steady production of flies from the stream during June–October.

Oviposition Behaviour.

Smart (1934) and Zahar (1951) record the occurrence of egg masses of *S. ornatum* placed immediately above the water-level on vegetation trailing in fairly

rapid water. In the present work, quantitative study of oviposition activity was attempted, using sticky traps as described by Broadbent & others (1948), consisting of metal cylinders 15 cm. diam., 30 cm. in length and covered with a detachable plastic sheet coated with commercial tree-banding grease. The sticky traps were supported horizontally over the stream so that one side of the cylinder was about 5 cm. above the water surface, and three such traps were operated from May to November 1952. All three were placed within a typical stretch of stream 4 metres long, selected for study, and where the main substratum for oviposition consisted of the leaves of the grass, *Glyceria* sp., trailing into relatively rapid flowing water. The adhesive-coated covers of the traps were changed at weekly intervals, except when information on diurnal changes in oviposition activity was required, when more frequent changes were made. It was soon found that the traps caught many female blackfly. Small numbers of females of *S. aureum* were caught but the following figures refer to *S. ornatum* only.

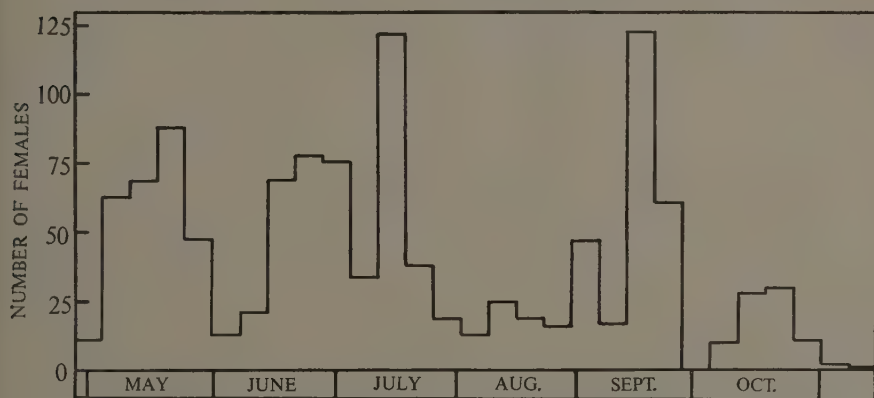


Fig. 2.—Number of gravid females of *S. ornatum* caught per sticky trap per week, 1952.

The catch of females of *S. ornatum* consisted entirely of fully gravid individuals together with a small number of males of the same species. The numbers of gravid females caught per week in 1952, plotted in fig. 2, is the mean of the three traps used. It will be noted that females were caught in varying numbers in each week from late April to early November except for the week 27th September–3rd October when heavy rain temporarily caused the traps to be submerged. Weather conditions in the district in 1952 were close to long-term averages so that it was not an exceptionally favourable blackfly season, a conclusion supported by comparison of fly numbers taken on cattle near the stream in 1952 with comparable figures obtained in 1954 (p. 422). The long season over which gravid females of *S. ornatum* occurred in 1952 would appear to be normal for the species.

During the second half of the 1952 season, the vegetation trailing into the stream along the 4-metre stretch on which the sticky traps were situated was carefully searched at least once a week and all leaves seen to be carrying eggs of *S. ornatum* (Zahar, 1951) were collected and preserved in alcohol. In the laboratory the egg-masses were removed from the leaves and freed from foreign matter. The combined egg-masses for each week were dried at 30°C. to constant weight and the weight recorded. The whole complement of eggs dissected from four gravid females and similarly treated were found to average 0.7 mg. dry weight per female, and this figure was used to obtain an estimate of the

numbers of females responsible for the eggs collected in the field. The results, together with the sticky-trap catches for the corresponding weeks, given in Table I, indicate heavy egg-laying activity in mid-August and mid-September, and show some remarkable changes in the amount of activity in successive weeks, *e.g.*, the weeks, commencing on 6th and 13th September, when approximately 140 and 1,390 females, respectively, laid within the 4-metre length. This short-term fluctuation was at least in part caused by weather differences, the latter

TABLE I.

Number of females ovipositing on a 4-metre length of the stream during the latter half of 1952 season.

Week beginning	No. of females ovipositing (estimated from weight of egg-masses)	No. of females caught on sticky traps	Total females laying within the zone
9 Aug.	3281	76	3357
16 "	1450	57	1507
23 "	817	49	866
30 "	160	142	302
6 Sept.	91	50	141
13 "	1023	368	1391
20 "	296	183	479
27 "	—	—	—
4 Oct.	0	30	30
11 "	23	84	107
18 "	1	89	90
25 "	0	32	32
1 Nov.	0	9	9
8 "	0	2	2

week being far less windy than the former and thus more suitable for oviposition activity (see p. 411). The sticky traps caught a highly variable proportion of the total number of females ovipositing on the 4-metre stretch of stream. During the first four weeks (Table I) the sticky traps caught about 2, 4, 6 and 47 per cent., respectively, of the total. Direct observation showed that traps caught only those females that attempted to oviposit within a few centimetres of the trap. The "attractiveness" to ovipositing females of the narrow zone around each trap, relative to the remainder of the 4-metre length of stream, presumably varied from one week to another. This variable "attractiveness" may be explained in part by the highly communal ovipositing habits of this species, where up to 20 or so females may oviposit on one grass leaf while leaves lying 10 cm. away under apparently similar conditions are ignored. The presence of ovipositing females or of eggs seems to act as an attractant to further gravid females in search of sites, and if the first females to arrive select a site outside the zone of a sticky trap, the latter may have a very low catch in spite of heavy activity a few centimetres away. Much of the fluctuation in sticky-trap catches shown in fig. 2 may have been due to such events and the numbers caught did not illustrate weekly changes in the numbers of females laying eggs in the 4-metre length of stream as a whole.

Ovipositing females were found to be active only in the period between sunset and dusk. The numbers taken on three sticky traps at hourly intervals on two typical evenings are given in Table II. As soon as the sun had set, large numbers of gravid females appeared flying within 1-5 cm. of the water surface over the rapid parts of the stream, settling and ovipositing on trailing vegetation or sometimes on stones or sticks protruding from rapid flowing water. Egg-laying activity

rapidly decreased as darkness fell, about one hour later. No gravid females were ever collected on sticky traps between onset of darkness and 10.00 a.m.-12 noon the following day, and no ovipositing females were seen outside the period from shortly before sunset to the onset of darkness. The presence or absence of wind appeared to have a great effect on oviposition. In May 1952, on four evenings

TABLE II.

Examples of timing of oviposition activity, 1952.
Number of gravid females caught on 3 sticky traps.

Period (G.M.T.)	23 May	24 May
12 noon — 5.00 p.m.	0	0
5.00 — 6.00 p.m.	6	2
6.00 — 7.00 p.m.	1	1
7.00 — 8.00 p.m.	38	79
8.00 — 9.00 p.m.	9	6

Effective sunset at trap site on above days, approximately 7.00 p.m.

when the wind exceeded about 10 m.p.h., no gravid blackflies were caught on the sticky traps, while on 14 calm or almost calm evenings many were caught on each evening.

Seasonal Variation in the Size of Females.

Edwards (1920) records that females of *S. (E.) equinum* (L.) obtained in the spring were larger than those obtained in the summer and that this was true to a lesser extent of those of *S. ornatum* also. In the present work, wing-length measurements, as an index to body size, were made on gravid females of *S. ornatum* caught on sticky traps. The right wings were removed, mounted in balsam, and measured by projecting the image on to a calibrated screen. The flies bred from pupae in late August (Table III) are seen to be appreciably bigger

TABLE III.

Seasonal variation in wing length of females, 1952.

Gravid females caught on	Mean wing length and S.D. (mm.)	No. of specimens measured
5 May	3.20 ± 0.19	39
14 "	3.21 ± 0.19	39
28 "	3.03 ± 0.23	86
21 June	2.72 ± 0.21	50
26 July	2.26 ± 0.20	44
23 Aug.	2.66 ± 0.21	50
20 Sept.	2.76 ± 0.18	50
25 Oct.	3.04 ± 0.28	46
Females bred from pupae on 26 Aug.	3.05 ± 0.11	46

than those ovipositing at about the same time and this is presumably a reflection of the time-lapse between emergence and oviposition. That is, the blackflies bred from pupae in late August were of a size approximating to those ovipositing much later in the season. It would appear from the above figures that large spring flies, ovipositing in May and which could only have been derived from overwintered larvae, gave rise to smaller flies ovipositing in June and July, but that the August-hatched flies were again of a size comparable with the spring flies. No explanation can at present be offered for these size changes, which appear in 1952 to have involved the production of undersized flies during early summer or late spring only.

Activity of Female Flies on Cattle.

Adult blackflies were collected as they landed on cattle grazing within 0.4 km. of the stream. A "pooter" consisting of a strong glass tube 15 cm. long and 2.5 cm. in diameter fitted with an inverted glass cone at one end was used. The cone was pierced by a hole about 3 mm. diameter through which the flies were sucked on holding the cone end over them. The suction was applied by the collector through a tube piercing the rubber cork closing the end of the glass tube opposite to that bearing the cone. With practice, it was found that one person could collect, from one cow, each fly as it landed, and that negligible numbers of flies were missed. The effect of the presence of the collector on the size of the catch appeared to be small. When he retired to a distance of 5 metres from the cow, blackflies were observed to land in numbers comparable with those obtained when the collector stood beside the cow. No examples of *S. ornatum* were ever observed to land on the collectors themselves. Since it was not practicable to use tethered cows, the blackfly numbers given in the following pages were obtained from cattle that moved about the pasture in a normal manner during each period of observation. This mobility of the cattle undoubtedly introduced an additional variable and thus increased the difficulty of analysis. The fly figures, however, do have the merit of having been obtained on cattle under normal conditions of free movement.

Two 18-month-old Ayrshire heifers were used in 1952, while three Shorthorn heifers, 2 years old, were used in 1953-54, two in 1953 and one in 1954. The standard procedure was to capture all blackflies as they landed during a collecting period of 15 min., at the end of which the flies were killed and tubed, and wet- and dry-bulb temperatures taken with a whirling psychrometer held in the shade at 0.5 metre above the ground. A further catching period was then begun. General notes of the weather conditions were made, particularly of wind conditions. On certain occasions wind-speed readings were taken with an air meter, and details of the procedure used are given on p. 417. For particular purposes fly-collecting periods of longer or shorter duration than the standard period of 15 min. were used, and details are given in the relevant sections.

Sites of Activity on the Cattle.

Blackflies landed almost exclusively on the under-surfaces of the cattle, and the very few flies which occasionally landed on the back, flanks or legs invariably took wing again to re-alight on the under-surface. Although flies landed at any point on the ventral side, most landed within 20 cm. of the navel, and many landing outside this area walked towards it or took wing to re-alight within it. Of the flies landing, those which commenced engorgement usually attached themselves in the navel region, particularly on the navel itself. Smaller numbers engorged on the udder and teats, and on the mid-line of the thorax forward to and including the dew-lap or brisket.

Breev (1950) records a similar spatial distribution of blackfly landing on

reindeer and has shown experimentally that the concentration of landing on the under-surfaces is caused by the tendency of the insects to settle on the less brightly illuminated parts of the body.

The fact that blackflies landed only on the ventral surfaces made it practicable for one collector to catch very nearly all flies as they landed, since he was able to keep all the area of landing under continuous observation during a collecting period.

Diurnal Pattern of Activity.

All times given in the following account refer to Greenwich Mean Time, and dawn and dusk times mean the earliest and latest times, respectively, at which it was light enough to see blackflies on the under-surfaces of the cattle with unaided sight.

TABLE IV.

Pattern of landing activity, early morning.
Number of flies landing on cow in 15-min. period within the hour
commencing at the time stated.

Date	Time of dawn	Time, a.m.						
		4-00	5-00	6-00	7-00	8-00	9-00	10-00
17 Aug. 1952	4.15	0	6	1	6	17	3	3
23 Aug. 1952	4.25	0	4	3	15	1	—	—
11 Aug. 1953	4.10	6	1	6	15	3	6	6

In early morning, blackflies began to arrive at the cows soon after dawn (Table IV) and close examination of the cattle at this time never revealed any fresh bites (recognised because the skin abrasions made by *S. ornatum* often continue to bleed for some time after the fly has left) suggesting that biting during the dark hours is absent or very rare. Some hours after dawn, blackfly landing usually increased to a small peak varying in time from day to day, and then decreased again. This pattern was obtained on each of five mornings' observations of which three examples are given in Table IV and agrees with the observations of D. M. Davies (1952) on *S. venustum* Say.

TABLE V.

Patterns of landing activity, mid-morning to dusk.
Number of flies landing on cow in 15-min. period within the hour
commencing at the time stated.

Date	Time											Time of dusk
	9.00	10.00	11.00	12.00 noon	1.00	2.00	3.00	4.00	5.00	6.00	7.00	
15 Aug. 1952	4	0	3	2	0	1	0	0	1	2	51	7.45
1 Sept. 1954	27	28	6	3	0	48	87	38	72	69	—	6.45

During the remainder of the day, two patterns of activity were discernible. On warm days with light cloud and large temperature and humidity changes, landing was low or absent until late evening when a sudden large peak occurred during the last hour of daylight. On cloudy days with smaller temperature and humidity changes the numbers of blackflies landing did not show a regular pattern and the late evening peak was often absent or ill-defined. The total number landing was often much greater on such days than on warm and less cloudy days. One example of each of the two types of pattern is given in Table V. Similar patterns of landing activity are recorded for *S. venustum* on man in Canada by D. M. Davies (1952).

While the cattle moved freely over the pasture during each fly-collecting period, a fairly well marked effect of the position of the cow on the number of flies landing on it was noted. When a cow approached a hedge or other cover a sudden increase in fly numbers was often noted both on calm and windy days. For example, of 45 flies landing between 6.00 and 7.00 p.m. on 16th August 1954, in calm conditions, 39 landed between 6.15 and 6.21 p.m. when the cow was adjacent to a hedge, and the remaining 6 during the rest of the period when the cow was in mid-field. Even when the cow remained virtually stationary in mid-field, large fluctuations in fly landing numbers sometimes occurred during short time-intervals as the following example shows (2nd September 1954):

2.50-3.00 p.m. 3; 3.10-3.20 34; 3.30-3.40 14.

Temperature and Landing Activity.

The geometric mean of (No. of blackflies + 1) landing per 15 min. was calculated for each degree Centigrade of the temperature range encountered in the 1954 observations (Table VI). Since wind speed has an important effect on landing activity (p. 417) separate geometric means are given (a) for observations made when the wind was less than about 5 m.p.h. and (b) for observations made at a greater wind speed. In each case, in addition to means based on the actual or uncorrected fly numbers obtained, a separate mean has been calculated from the fly numbers corrected for seasonal variation in fly abundance. The correction factor for abundance was obtained as follows. The mean maximum

TABLE VI.

Geometric means of (No. of flies + 1) landing on a cow per 15 min. in 1954.
(Wind speed estimated.)

Temp. (°C.)	Wind absent or less than 5 m.p.h.			Wind more than 5 m.p.h.		
	Uncorrected	Corrected	No. of observations	Uncorrected	Corrected	No. of observations
10	14.4	13.8	6	—	—	—
11	13.8	21.9	6	14.4	8.7	4
12	6.4	6.9	17	2.6	2.2	8
13	13.5	14.1	22	3.7	2.9	11
14	12.6	12.9	24	2.4	3.2	6
15	9.1	12.9	14	2.6	2.7	6
16	17.4	21.4	14	3.9	4.9	13
17	11.2	12.3	15	1.6	1.8	18
18	12.3	9.1	18	1.9	1.8	9
19	13.2	11.5	14	1.4	1.4	6
20	6.4	8.3	8	3.7	7.8	2
21	16.2	15.1	11	4.1	3.8	3
22	25.7	16.2	8	—	—	—

number of flies landing per 15 min. during the last hour of daylight on calm evenings was calculated for each of five parts of the 1954 fly-season. The number of flies obtained is likely to be more closely related to fly abundance in late evening than during the day when the inhibitory effects of wind or too great an evaporation rate are likely to mask seasonal effects. Only data obtained on calm evenings were used. The correction factors were calculated as shown in Table VII and were then applied to all landing observations made within the relevant part of the season.

In Table VI it will be seen that in both corrected and uncorrected means there was no consistent tendency for landing activity of blackflies to increase with temperature or to be related directly to the temperature measured. Presumably the effect of temperature is complex, and is masked by other factors, particularly the variable caused by the mobility of the cows and the resultant position effect noted above (p. 414).

TABLE VII.

Correction factors used for variation in fly abundance, 1954.

Period	No. of evenings used	Evening activity peak. Mean max. no. flies landing/15 min.	Correction factor
Whole season ..	21	45	—
8-13 July ..	4	31	1.45
3-13 Aug. ..	8	19	2.37
21-26 Aug. ..	3	31	1.45
30 Aug.-2 Sept. ..	4	87	0.52
21-24 Sept. ..	2	119	0.38

Note. For whole season, mean max. no. flies landing /15 min. in evening peak = 45
During 8-13 July, mean max. no. flies landing /15 min. in evening peak = 31

The correction factor used for fly numbers obtained during 8-13 July = $45 \div 31 = 1.45$

Saturation Deficiency and Landing Activity.

In fig. 3, c, the mean log of (No. of flies + 1) landing on the cow in 15 min. is plotted against saturation deficiency (mm. Hg.). Only observations made at less than an estimated wind speed of 5 m.p.h. are included. In both corrected and uncorrected means it is seen that landing was remarkably constant over the humidity range except for the ill-defined peak at 3 mm. Hg. saturation deficiency involving an increase from about 10 flies per 15 min. at 2 and 4 mm. to about 20 flies at 3 mm. Hg. saturation deficiency (geometric means).

In order to test whether this peak was fortuitous, the observations made in the first half of the season (July-mid-Aug.) were treated separately from those made in the second half (mid-Aug.-Oct.). The uncorrected and corrected means are plotted in fig. 3, a and b. The slight peak at 3 mm. saturation deficiency is apparent in both halves of the season and suggests that the greater landing found at that humidity was a reproducible result.

In the uncorrected data (fig. 3, a) the means for the second half of the season are seen to be consistently higher than those for the first half. This agrees with

the impression gained during the field work that blackflies were considerably more abundant in the late part of the season than during the first part. The corrected means for the two halves of the season (fig. 3, b) agree much more closely and thus show that the corrections for changes in blackfly abundance during the season (Table VII) did do so to some degree, and that the number of flies in the evening peak was related to fly abundance as would be expected.

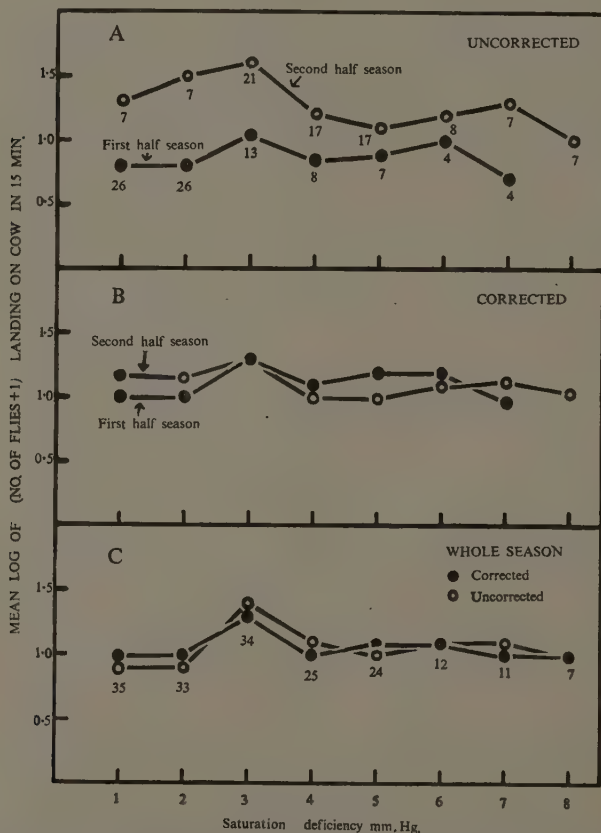


Fig. 3.—Blackfly landing in relation to saturation deficiency. Only observations made when wind was less than 5 m.p.h. are included. The figures below each point give the number of observations on which it is based.

Corresponding figures for observations made when the wind was estimated at over 5 m.p.h., plotted against saturation deficiency in fig. 4, seem to confirm those in fig. 3, for lower levels of blackfly activity.

Figs. 3 and 4 show that variations in saturation deficiency over the small range encountered in this work had relatively little effect on the number of blackflies landing. Since the number of flies landing during a given period formed an

unknown proportion of the flies on the wing at the same time, and since humidity may differentially affect both flying and landing (see D. M. Davies, 1952) this result is perhaps not unexpected.

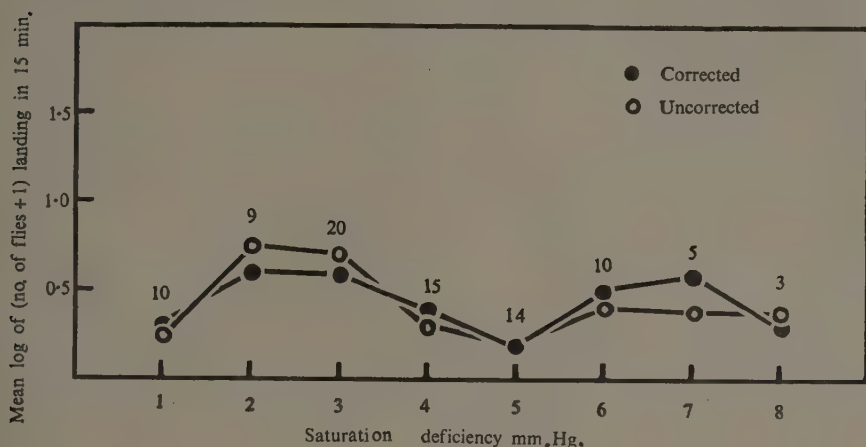


Fig. 4.—Blackfly landing in relation to saturation deficiency. Observations at more than 5 m.p.h. wind speed only. Figures above each point give the number of observations on which it is based.

Wind and Landing Activity.

Throughout the work it was clear that landing activity was markedly reduced or absent on windy days, and it was repeatedly observed that the number of flies landing decreased rapidly when a strong breeze suddenly arose after a calm period, and *vice versa*.

In 1952 and 1953 a number of measurements of wind speed were made with an air meter held into the wind at 0.5 metre above the ground and within a few metres of the cows used as fly attractants. Immediately after a 15-min. fly-catching period, 10 readings of wind speed for a duration of 30 seconds were made, each separated by about 1 min., and the mean wind speed was calculated. The results are summarised in Table VIII and show that when wind exceeded an

TABLE VIII.

Wind speed and landing activity. Number of 15-min. periods when fly landing was present or absent.

Wind absent or less than 5 m.p.h.		Wind more than 5 m.p.h.	
Flies landing	No flies landing	Flies landing	No flies landing
1952-53 Observations (wind speed measured by air meter)			
38	5	3	20
1953-54 Observations (wind speed estimated)			
187	7	60	38

average of 5 m.p.h., fly landing was usually completely inhibited in these particular observations.

A difficulty in using an air meter soon became apparent. The cow being used for fly-landing counts frequently moved in or out of the shelter of a hedge during a collecting period, making it impossible to arrive at a figure for the average wind speed to which the cow had been exposed that would have any meaning. During the remainder of 1953 and the whole of 1954 no air-meter readings were taken, but an estimate was made of the wind speed to which the cow was exposed during the catching period, in the light of experience of wind of known strength gained from the earlier air-meter observations. Observations were divided into two wind-speed categories, (1) wind absent or less than about 5 m.p.h. and (2) wind more than about 5 m.p.h. The number of collecting periods in which landing was absent or present in the two categories are given in Table VIII. The greater frequency of landing at more than 5 m.p.h. estimated wind speed (60 periods out of 98) compared with the air-meter observations (Table VIII, 3 out of 23) suggests that in the former case the observer tended to over-estimate the wind speed.

In Table VI (p. 414) the geometric means of the (No. of blackflies landing + 1) per 15 min. at each temperature are given for the two estimated wind categories listed above. It will be seen that the mean number landing when the wind speed was more than about 5 m.p.h. was usually about a quarter of what it was when wind speed was less than that figure, at the same temperature.

A comparable reduction of landing activity when wind speed was more than about 5 m.p.h., as compared when it was absent or lower than this, is apparent from an analysis of figures obtained in 1952 when two cows were separately observed (Table IX). In this Table the mean fly numbers are seen to be much

TABLE IX.

Geometric mean of (No. of flies + 1) landing per 15 min. in 1952.

	Wind absent or less than 5 m.p.h.	Wind more than 5 m.p.h.
Cow no. 1 ..	6.8 (53)	1.4 (16)
Cow no. 2 ..	4.6 (38)	1.3 (12)

No. of observations given in brackets.

lower than those obtained in 1954 (Table VI). This difference agrees with the impression gained that in 1954 blackflies were far more abundant throughout the season than in 1952.

The reduction of fly-landing numbers caused by wind is further illustrated by comparing the figures for a windy day with those for a day of light breeze (20th and 22nd September, 1954). The days are close enough together for changes in fly abundance to have been negligible and the temperatures and saturation deficits encountered were very similar. Flies were collected during identical time periods on the same cow during the two days. On the windy day 61 flies were collected in 70 min. while during the same period on the day of slight wind 531 flies were taken.

Length of Time spent by Blackflies on a Cow.

Of the total number of blackflies landing on a cow during a given period only a small proportion attempted to bite (see p. 420). The length of time taken to engorge was observed in 15 cases. After they had landed, these flies spent a

variable period, up to at least 10 min., in climbing the belly hair of the cow. After attaching themselves to the skin, ten of the flies became fully engorged in 6-12 min. The remaining five blackflies took 14-18 min. to engorge but did not show signs of abdominal swelling for the first 8-10 min., presumably because they experienced difficulty in causing the skin abrasion, produced by *Simulium* mouthparts, to bleed. In the ten flies which did not show delayed engorgement, the abdomen was noticeably swollen after 3 min. and shortly after that time a drop of liquid appeared at the tip of the abdomen, indicating that concentration of the blood-meal had commenced and that fluid was being eliminated.

Indirect information on the length of time spent on the cow by all the blackflies landing was obtained by the following method. Flies were collected as soon as they landed on the cow for alternate periods of 10-min. duration (e.g., 10.00-10.10, 10.20-10.30, etc.). During the intervening 10-min. periods (10.10-10.20, 10.30-10.40, etc.) no flies were taken. Thus each 10-min. collecting period was followed by a non-collecting period of similar duration. At the start of each collecting period any flies which had already landed on the cow were quickly caught and counted. These flies must have landed at some time during the preceding 10-min. non-collecting period. The total number of blackflies that landed during, say, six successive alternate 10-min. collecting periods was thus known. From this known figure an estimate of the number of flies that had landed during the non-collecting periods, which would in this particular example be five in number, was calculated, assuming that flies landed during the non-collecting periods on average at the same rate as during the collecting periods. Thus if 60 blackflies landed during six collecting periods, the calculated number landing during the non-collecting periods would be 50. Provided that the sum total of flies for several successive alternate collecting periods are used, the above assumption seems justified. The number of flies present at the end of each

TABLE X.

Proportion of "flies missed" at different levels of blackfly activity.

Expt. no.	Activity level, flies landing per 15 min.	(a) Estimated no. of flies landing during non- collecting periods	(b) No. of flies taken at ends of non-collect- ing periods	Estimated no. of flies missed (a-b)	χ^2	P
1	low, 19	90	31	59 (65%)	8.34	<.01
	high, 51	241	45	196 (81%)		
2	low, 21	71	33	38 (53%)	15.95	<.01
	high, 57	189	39	150 (78%)		
3	low, 9	19	10	9 (47%)	10.26	<.01
	high, 34	69	10	59 (85%)		
4	low, 16	34	9	25 (73%)	3.03	>.05
	low, 21	71	33	38 (53%)		
5	low, 18	36	11	25 (69%)	0.49	c.50
	low, 27	71	31	40 (56%)		

non-collecting period was known, and these figures were summed. Thus if the calculated total number of flies that landed during five non-collecting periods (estimated as above) was 50, and a total of 15 flies was present on the cow at the ends of the periods, then 35 flies are calculated to have landed on the cow and departed again within less than 10 min. These flies are referred to below as "flies missed".

The proportion of flies missed when fly activity was low was compared with the proportion missed when activity was high (see Table X). In experiments 1-5 the two periods compared were on the same day, and the same cow was used throughout. It will be seen that in experiments nos. 1-3, a significantly greater proportion of blackflies were missed when activity was high than when it was low. This result is interpreted as meaning that when blackfly landing activity increased, the flies tended to spend less time on the cow, leading to a higher proportion being missed. It is perhaps to be expected that the combination of environmental factors stimulating flying activity, leading to more flies reaching the cow, would also tend to keep the flies restless and shorten the period spent on the cow. Experiments 4 and 5 (Table X) show that the proportion of "flies missed" sometimes changed even when activity remained relatively constant, although the differences between the compared periods in these two experiments are not statistically significant. D. M. Davies (1952) has shown, in *S. venustum*, how variation in the rate of change of barometric pressure affects the proportion of blackflies that bite amongst those that landed, and in the present results a similar effect may have been at work. These variations in the average length of time spent by flies on the cow may well have been a reflection of changes in biting-activity.

If the results of the five experiments (Table X) are summed, it is found that of a calculated total of 891 flies landing on the cow, 639 or 71 per cent. were missed. At least 70 per cent. of all the flies landing therefore spent less than 10 min. on the cow before departing.

Proportion of Flies Landing that Attempted to Bite.

It proved impossible to observe directly the proportion of flies landing during a given period that stayed to bite during the same period. This was caused partly by the fact that engorging flies were hidden by the belly-hair and therefore difficult to count. Indirect observations showed that only a relatively low proportion of flies landing attempted to bite. Thus, in 50 min., on 25th August 1953, ten blackflies were seen to become attached to one cow, which was carefully examined, while during the same period a second collector obtained 98 blackflies as they landed on an adjacent cow. In 45 min., on 24th August 1953, 11 engorged flies were obtained from a cow, and a further four such flies were lost because they detached themselves on completing engorgement at the same time as another fly was being tubed. Probably no more than a further two flies engorged without being observed, so that the total was about 17 engorgements in 45 min. During the succeeding 15 min., when fly activity remained much the same as previously, 41 females landed on the same cow.

Further information on the number of blackflies biting was obtained in the following way. All flies were collected for alternate periods of 10 min. as they landed on the cow, while the flies which landed on the cow during the intervening 10 min. were not collected as soon as they landed, and thus had opportunity to bite. The total number which landed on the cow during several such non-collecting periods was estimated as being equal to the total number which landed during the collecting periods which alternated with them. Details of the method are given on p. 419. It seems likely that this estimate of the number landing during the non-collecting periods is reasonably accurate if the numbers for several successive collecting periods are summed, since the errors involved in each

non-collecting period would tend to cancel each other out in the total for, say, ten or more periods. Of the blackflies landing during the non-collecting periods, the proportion that had bitten the cow was found by determining the number which contained fresh blood in the mid-gut by dissection. The non-collecting period of 10 min. was selected as being short enough to prevent flies engorging and departing before the next collecting period started.

TABLE XI.

Number of flies containing blood in relation to number landing, 1954.

Date	No. of 10-min. periods used	Calculated total no. landing	No. containing fresh blood	% containing fresh blood
31 Aug. ..	14	127	9	7.1
1 Sept. ..	25	549	35	6.3
2 " ..	20	406	15	3.7
20 " ..	7	55	7	12.7
21 " ..	9	297	21	7.1
22 " ..	10	c.430	43	10.0
23 " ..	9	137	11	8.0
24 " ..	8	118	11	9.3

The results for eight days in 1954, obtained by the above method, are given in Table XI. It will be seen that the proportion of flies that landed and contained fresh blood varied on different days from 3.7 to 12.7 per cent. These percentages are almost certainly lower than the true proportion that would have bitten, since flies were allowed to bite only during alternate 10-min. periods. It was observed (p. 419) that flies that eventually bit the cow usually spent some minutes in climbing the hair of the cow and in reaching and probing the skin before engorgement commenced. Thus with the present method, only those flies that landed during the first 5 min. or so of the 10-min. non-collecting period would have had time to begin engorgement. Those that landed during the last 5 min. would probably not have had time to commence engorgement before the next 10-min. collecting period began. The proportion of flies biting on each day (Table XI) recorded by the above method, therefore represents probably about one-half of the true biting rate. Thus the range of from 3.7 to 12.7 per cent. of landing blackflies biting on different days probably represented a true biting proportion of the order of twice this, i.e., 8 to 25 per cent. These proportions indicate the generally low number of flies biting, compared to the number landing, in this species. The above figures can be compared with those obtained by D. M. Davies (1952) for *S. venustum* on man, where the $\sqrt{\text{biting}}/\sqrt{\text{landing}}$ ratio varied from about 0.4 to 0.5, representing a proportion of 16-25 per cent. of landing flies actually biting.

Of the blackflies landing on the eight days of observations, the differences in the proportion that bit (Table XI) have been examined by the χ^2 test, and this indicates that a significantly greater proportion of the flies bit on 22nd September than on 2nd September ($P = < .01$).

The Nuisance caused by *S. ornatum* to the Cattle.

From the figures of blackfly landing per unit time in 1952-54, it is estimated that the number of blackflies landing per cow per day between April and October in the three seasons was:—

1952, 100-500; 1953, 300-2,000; 1954, 500-4,000.

The lower figure in each case is an estimate for a day of less suitable weather, and the higher for a day highly suitable for blackfly activity. If a figure of 16 per cent. of flies landing is considered to have bitten (mean of extremes of 8 and 25 per cent. estimated from Table XI), the estimated number of bites per cow per day becomes:—

1952, 16-80; 1953, 48-320; 1954, 80-640.

Since bites are restricted largely to the navel area, the mid-ventral line of the thorax and the udder and teats, the above numbers of bites per cow per day might have been expected to constitute a considerable nuisance to the cattle. In fact they appeared to suffer no appreciable ill-effects although thick scar tissue covered the navel itself and part of the mid-line of the thorax. After a day of heavy fly activity some bleeding took place around the scar areas, but no suppuration was observed throughout the three seasons' work.

Individual cows showed great variation in their ability to remove engorging blackflies by using their tongues and this possibly partly accounted for the absence of ill-effects caused by bites. One cow invariably licked off all attached flies within one or two min. of attachment, and she probably swallowed most of them, while another cow very rarely used her tongue.

Fresh bites of *S. ornatum* were frequently visited by Muscid flies, which lapped up exuding blood. *Hydrotaea irritans* (Fall.) and *H. meteorica* (L.) were particularly involved in this but their numbers did not appear high enough to cause serious effects. Other biting insects visiting the cattle included very large numbers of *Culicoides* spp., moderate numbers of *Anopheles claviger* (Mg.), *Aedes annulipes* (Mg.) and smaller numbers of *Stomoxys calcitrans* (L.) and *Haematobia stimulans* (Mg.).

Discussion.

Periodic sampling of the aquatic stages during one season showed that adults of *S. ornatum* were produced in large numbers from April to October, except for a short gap in mid-May when emergence of those derived from overwintered larvae had ended and emergence of the succeeding generation had not yet begun. Correlated with this extended fly-production period of about seven months, ovipositing females were trapped in the same season from May to early November. These results agree with those of Smart (1934) on the same species, and afford a strikingly different picture from that obtained by D. M. Davies (1950, 1952) for *S. venustum* in Canada. Adults of the latter species were abundant between mid-May and mid-July, a period of only about two months, while other species studied by D. M. Davies had an even shorter emergence period. *S. ornatum* would appear to have an exceptionally long emergence period for a blackfly of temperate latitudes.

The restriction of oviposition activity in *S. ornatum* largely to the period between sunset and dusk agrees with the results of Wu (1931) on several species and of Fredeen, Rempel & Arnason (1951) on *S. arcticum* Mall. The sudden appearance of many gravid females of *S. ornatum* over the stream at sunset suggests that many rested in situations close to the stream awaiting suitable conditions, as in *S. arcticum*. Despite considerable search by sweep-netting in

the present work, resting gravid females were not found. Close observation of ovipositing females on many evenings did not disclose that any succumbed to predators such as species of EMPIDAE which were always numerous. Females that had completed egg-laying did not seem exhausted but took wing without delay, so that the observational evidence, for what it is worth, suggests that many flies survived for an unknown period after laying eggs. This suggestion is supported by the finding that a high proportion of females attracted to cattle have already undergone at least one complete gonotrophic cycle (L. Davies, 1955). The few male *S. ornatum* caught on sticky traps at the same time as gravid females suggests that some females copulate again after egg-laying, although this was not directly observed.

The failure to detect any marked effect of air temperature and saturation deficiency on the number of *S. ornatum* landing on cattle, despite the occurrence of a diurnal pattern in landing numbers correlated broadly with environmental changes, particularly on warmer days, may have been caused by several factors. The cattle used were allowed free movement and the changing position of the cattle would be expected to contribute to the effect of other variables, such as light-intensity changes, in masking any temperature and humidity effects. Furthermore, the temperature range and particularly the humidity range encountered may have been too small to reveal their effects on landing numbers. Landing activity was, however, very markedly affected by wind speed. The present work showed that winds of above 5 m.p.h. were sufficient to reduce considerably the number of *S. ornatum* landing on cattle. This species would appear to be more sensitive to the effects of wind than is *S. venustum*, in which a wind of about 15 m.p.h. is required to produce a similar effect (D. M. Davies, 1952). Since both blackflies are of much the same size, this difference suggests that *S. ornatum* is more susceptible to desiccation than *S. venustum*, in view of the probability (D. M. Davies, 1952) that the effects of wind on blackflies at these relatively low speeds seem to be through its effect on evaporation rates.

The results of Berzina (1953) on the effect of wind on blackfly activity differ somewhat from those recorded in the present paper. She found that wind had little effect on the activity of *S. erythrocephalum* (Deg.) in the Volga delta, and her studies on several species, including *S. ornatum*, in arctic Russia showed that wind depressed the landing activity on man at unfavourable temperatures, and stimulated it at optimum temperatures. Berzina encountered a greater temperature range in her work than was the case in the present study, where possibly most of the smaller range fell into what may be less favourable temperatures for activity of *S. ornatum*.

Alternation of collecting periods with non-collecting periods of similar duration showed that a high proportion of the blackflies landing on cattle departed again within less than 10 min. This result fits in with the fact that even when landing activity was heavy the flies did not show a tendency to accumulate and form a swarm around the cattle. When all blackflies were collected as they landed for several hours, the numbers taken did not show a consistent tendency to fall off over a shorter or longer period. This suggests that the blackflies arriving at cattle over several hours constituted a stream of individuals, each spending a short time on the animal and then being replaced by others. This fits the facts better than the idea that the same individuals tend to visit a cow several times during the same day and remain in the vicinity of the cattle for long periods. A study of the age-composition of blackfly catches made on cattle at different times of the day (L. Davies, 1955) suggests strongly that flies landing at one time of day are largely different individuals to those landing at other times of the same day, and supports the idea of a continuous stream of flies representing the individuals which became activated for a relatively short period and then return to their unknown resting places.

Summary.

Samples of aquatic stages, taken from a small lowland stream near Durham, England, showed that the blackfly, *Simulium ornatum* Mg., emerged in quantity from April to October (7 months) and that oviposition took place between May and October. Oviposition was confined to the period between sunset and dusk.

Flies emerging in the early summer of 1952 were smaller than those emerging in the spring and late summer of that year.

S. ornatum landed on cattle from dawn to dusk, with usually a small peak 2-4 hrs. after dawn, and a large peak between sunset and dusk on warm, sunny days. On cool, cloudy days the number of flies landing showed irregular fluctuations throughout the day.

The number of flies landing on untethered cattle was not markedly dependent on air temperature or saturation deficiency. Winds of over 5 m.p.h. markedly decreased or inhibited landing activity.

On average about 70 per cent. of all blackflies landing spent less than 10 min. on cattle after landing, and this interval sometimes decreased as the total number of flies landing per unit time increased.

Of the flies which landed, some 8 to 25 per cent. were calculated to bite the cow, and the proportion which bit varied significantly from day to day. The number of bites sustained by cattle in the district appeared to cause no ill-effect, apart from the formation of scar tissue in the navel region where most bites were inflicted.

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FIELD TRIALS OF LARVICIDES AGAINST *CULICOIDES* WITH A DISCUSSION ON THE RELATIONSHIP BETWEEN RAINFALL AND LARVAL CONTROL.

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Field trials of various insecticides against larvae of the biting midge, *Culicoides impunctatus* Goetgh., were conducted on Soutra Hill near Edinburgh in the winter of 1953-54. Results have been reported in detail by Kettle, Nash & Hopkins (1956). It was found that the immediate effect of applying insecticides to the breeding sites was disappointing—the larval mortality bearing little relationship to the dosage of insecticide applied. A more constant picture was obtained when the residual effect was determined. It appears that the insecticides may not kill larvae already present in the soil but they prevent re-infestation of the breeding site. Consequently in the additional trials reported here the assessment was made solely on the residual effect.

In the earlier trials the necessary dosage of insecticide for each plot was applied in 14 pints of liquid. As each plot had an area of 100 square yards (10 yd. × 10 yd.) this was equivalent to the application of 80 gallons per acre. The practical problem presented by such a high volume/area ratio became evident during a full-scale field trial at Loch Maree in Wester Ross in the spring of 1955. The modern trend in large-scale spraying programmes is towards low-volume spraying, e.g., five gallons per acre. This development has been made possible by the production of nozzles which achieve good coverage at low volume. It was obviously essential to know whether low-volume spraying was suitable for midge control, and trials were therefore conducted on Soutra Hill to supply information on this point.

Two separate sites had been utilised previously for experimental purposes on Soutra. Larval sampling in the autumn of 1954 had shown that some of the earlier treatments, applied in 1953-54, were no longer exerting any larval control; indeed a few had never been effective. It was convenient to re-use these plots since their larval history was known for the past two years.

Methods.

Six plots on each site were sprayed at different volumes, namely 5, 10, 15, 25, 50 and 80 gals./acre. The insecticide used on all plots was a 50 per cent. DDT wettable powder. It was, in fact, part of the same preparation as was used earlier. Plots on site 1 received 50 mg./sq. ft. of the p,p' isomer and those on site 2 25 mg./sq. ft. Three untreated plots on site 1 and two on site 2 were sampled as a check on any natural fluctuation in midge density. The spraying was carried out with a Four Oaks Ross knapsack sprayer. Five of the six spray volumes were obtained by using flat-fan type nozzles with the appropriate delivery. For the sixth, 80 gal./acre, a swirl-disc unchokable nozzle was used. It was essential, for satisfactory comparison, that the dosage in all plots within each site should be constant (Table I). To exceed the required volume would automatically increase the dosage and render comparisons invalid. In practice, it was found that the best method of measuring the volume of spray applied was to place a known volume, appreciably larger than that required, in the sprayer, to apply an amount of spray that was known, by experience with each particular nozzle,

to be approximately correct, and to measure the volume remaining in the sprayer after application.

Site 1 was sprayed on 29th April 1955 and site 2 on 30th June 1955. The effect of the treatment was estimated by larval sampling during the period 5th

TABLE I.
Actual dosages of p,p'DDT applied to experimental plots, 1955.

Volume of spray (gals./acre)	Dosage (mg./sq. ft.)		Percentage error			
	Site 2 25	Site 1 50	25		50	
			—	+	—	+
80	25	50		0		0
50	25	54		0		8
25	24	48	4		4	
15	25	45		0	10	
10	26	42		4	16	
5	26	50		4		0

Site 1 was sprayed on 29th April, site 2 on 30th June.

October to 7th November 1955. The sampling technique was the same as that used earlier. These samples were not taken at random within the plots but were randomised over those parts where the flora indicated suitable conditions for larvae of *C. impunctatus* (Table II).

TABLE II.
Dominant vegetation of samples taken from sites 1 and 2 where 51 and 36 samples, respectively, were taken on each occasion, 1955.

SITE 1						
Sampling occasion	<i>Sphagnum</i>	<i>Polytrichum</i>	<i>Polytrichum</i> and <i>Sphagnum</i>	No moss	Total with <i>Sphagnum</i>	Total with <i>Polytrichum</i>
1	23	8	18	2	41	26
2	35	1	15	0	50	16
3	32	3	15	1	47	18
Total	90	12	48	3	138	60
Pre-treatment sampling total	99	12	42	0	141	54

SITE 2						
1	4	12	20	0	24	32
2	10	12	14	0	24	26
3	12	10	14	0	26	24
Total	26	34	48	0	74	82
Pre-treatment sampling total	18	44	46	0	64	90

It was decided to make further observations on those treatments in the earlier work (Kettle, Nash & Hopkins, 1956) which had shown marked residual activity in the previous autumn (1954). The treatments concerned were DDT dust, wettable powder and water-miscible concentrate at 50 and 200 mg. per sq. ft., γ BHC wettable powder and water-miscible concentrate at 200 mg. per sq. ft. These insecticides had been applied on site 1. On site 2, observations were made on DDT wettable powder, γ BHC wettable powder and chlordane water-miscible concentrate all at 50 mg. per sq. ft. and dieldrin wettable powder at 25 mg. per sq. ft. The results of these observations are presented on p. 428, and are followed by those of the new work on spray volumes.

Results.

One effect of the long hot dry period during the summer of 1955 was to reduce the larval population of *C. impunctatus*. As a result, in the autumn of 1955, the untreated plots on Soutra 1 and 2 showed decreases of 79 and 73 per cent., respectively, in their larval densities compared with the autumn of 1954. The overall reduction was 76 per cent., which was not evenly distributed. Thus on

TABLE III.

Number of larvae of *C. impunctatus* found on sampling plots two years after application of insecticide.

Site	Insecticide	Dosage (mg./ sq. ft.)	Larvae on sampling dates, 1955			Total larvae			No. expected 1955	Difference (%) + —
			5.x.55	14.x.55	28.x.55	Winter 1953-54	Autumn 1954	Autumn 1955		
1	DDT dust	50	0	0	0	20	2	0	109	99
"	" "	200	0	0	0	42	1	0		
"	DDT w.p.	50	0	0	0	27	1	0		
"	" "	200	0	0	0	28	0	0		
"	DDT w.m.c.	50	0	0	0	42	2	0		
"	" "	200	0	1	0	28	0	1	41	95
2	DDT w.p.	50	0	0	0	36	1	0		
1	BHC w.p.	200	0	2	0	20	0	2		
"	BHC w.m.c.	200	0	0	0	18	1	0		
2	BHC w.p.	50	0	0	0	46	2	0		
2	Chlordane w.m.c.	50	2	0	1	38	13	3	19	84
2	Dieldrin w.p.	25	1	0	0	72	8	1	35	97
1	Untreated		0	0	0	20	34	0		
"	"		0	3	1	30	55	4		
"	"		11	3	12	16	54	26		
2	"		2	0	0	16	46	2		
"	"		14	13	10	59	98	37		
Total untreated						141	287	69		

w.p. = wettable powder; w.m.c. = water-miscible concentrate.

Soutra 1 the three untreated plots showed larval reductions on 100, 93 and 52 per cent., and on Soutra 2 the two observations were 96 and 62 per cent. *C. impunctatus* had been virtually eliminated from three of the five untreated plots but had only been reduced to about half in the other two. In the light of this natural reduction it would be unwise to attach much importance to larval reduction in any single treated plot, but grouped data should be more reliable.

Prolonged residual effect of DDT, γ BHC, dieldrin and chlordane.

The earlier results and conclusions on these treatments have already been reported in Kettle, Nash & Hopkins (1956). In assessing the effect of these treatments the larval densities must be compared with the original pre-treatment densities (winter, 1953-54) and allowance made for the observed reduction in the untreated plots (Table III). The results can be expressed as the ratio of the number of larvae observed to the number of larvae expected if the natural reduction, mentioned above, had alone taken place.

Only one larva was recovered from the six DDT trials on Soutra 1 compared with an expected larval total of 91, which gives a ratio of 1:91. On Soutra 2 the result of the single DDT treatment was 0.18. The two γ BHC treatments on Soutra 1 yielded 2:19 larvae and that on Soutra 2, 0.22. Therefore DDT appears to be exerting a control of 99 per cent. (1:109 survival) and γ BHC one of 95 per cent. (2:41 survival). Similarly, chlordane achieved 84 per cent. control (3:19 survival) and dieldrin 97 per cent. (1:35 survival). It seems that all of these 12 "old" treatments were still effective nearly two years after application, but it must be remembered that, owing to exceptional climatic conditions, only two of the five untreated plots produced adequate numbers of larvae. So that, had the earlier insecticide treatments ceased to be effective, it would have been anticipated that two-fifths (i.e., 5) of the 12 "old" treated plots would have been re-infested. In the event none of them was re-infested.

It is of interest, but too much importance should not be attached to the fact, that the relative merits of the insecticides remain the same. On Soutra 1, DDT is still better than γ BHC (99% as compared with 90) and on Soutra 2, chlordane (84%) still lags behind the others, with dieldrin (97%) slightly less effective than either DDT (100%) or γ BHC (100%).

Effect of spray volume on insecticidal effectiveness.

The plots used on site 1 for the volume trials had received ineffective insecticidal treatment in the winter of 1953-54. In most of these plots the percentage increase in larval density after treatment was greater than in the untreated plots. The overall increase in the "treated" plots was +168 per cent. compared with +104 per cent. in the untreated.

The result of applying 50 mg. p,p'DDT/sq. ft. in different volumes of spray is given in Table IV. The most striking feature of these results is that in spite of the large natural reduction in midge density the effect of treatment is less marked than in the previous work, when only one concentration was used. The two trials in 1953-54 with the same DDT wettable powder and the same dosage (50 mg./sq. ft.) and at 80 gals./acre gave first-year residual controls of 96 and 97 per cent. (see Table III and Kettle, Nash & Hopkins, 1956, Tables IV and X), yet in the 1955 trial the corresponding control was only 20 per cent. (Table IV). When the six separate treatments at 50 mg./sq. ft. are grouped, the residual control is 43 per cent. Two treatments (15 and 25 gals./acre) were particularly successful, giving 81 and 86 per cent. control, respectively, but under the peculiar conditions of 1955 too much reliance should not be placed on single results.

On site 2, use was made of the plots which had been treated with malathion. This insecticide produced an immediate larval reduction of 55 per cent. but hardly any residual action. In the autumn of 1954, the larval density in the malathion-treated plots showed an increase of 17 per cent. over the pre-treatment density. If allowance is made for the 62 per cent. increase in the four untreated plots on site 2, recorded by Kettle, Nash & Hopkins (1956, Table X), then malathion was still exerting 28 per cent. control. Even so the effect of malathion was declining, as is to be expected with a hydrolysable organic phosphorus compound.

These plots on site 2 received 25 mg. p,p'DDT/sq. ft. The results are somewhat surprising. When the data are grouped, there is no residual control,

whereas in the earlier trials (Kettle, Nash & Hopkins, 1956, Table IV) the application in wettable powder of 3 and 12 mg./sq. ft. gave, respectively, 40 and 80 per cent. residual control. This is in agreement with the less effective control shown by 50 mg./sq. ft. in the 1955 trial. In the absence, from the grouped figures, of any larval control it is not possible to discuss the effect of spray

TABLE IV.

Effect of spray volume on the application of DDT wettable powder at 25 and 50 mg. p.p'DDT/sq. ft.

Site	Spray volume (gals./acre)	Dosage (mg./sq. ft.)	Larvae on sampling dates, 1955			Total larvae			No. expected 1955	Difference (%) + —
			5.x.55	14.x.55	28.x.55	Winter 1953-54	Autumn 1954	Autumn 1955		
1	5	50	2	10	2	18	71	14	17	18
"	10	50	0	9	2	13	50	11	12	8
"	15	50	3	0	0	22	66	3	16	81
"	25	50	0	3	0	58	93	3	22	86
"	50	50	1	4	1	11	33	6	8	25
"	80	50	6	9	5	34	105	20	25	20
Totals						156	418	57	100	43
2	5	25	5	2	1	45	46	8	11	27
"	10	25	6	4	2	43	54	12	13	8
"	15	25	8	5	9	56	52	22	12	83
"	25	25	2	0	6	30	92	8	22	64
"	50	25	5	3	0	58	36	8	9	11
"	80	25	6	5	7	34	32	18	8	125
Totals						266	312	76	75	1

Number expected based upon 1954 figures. For untreated plots see Table III.

volume on insecticidal activity. Nevertheless it is perhaps worth noting that at 80 gals./acre the larval density showed an increase of 125 per cent. over that expected.

It can be argued that the earlier malathion treatment was still producing 28 per cent. control in the autumn of 1954 and consequently the "pre-treatment" larval density for the volume trial was artificially depressed and should have been 431. When due allowance is made for this (giving a corrected total expected of 103), the application of 25 mg./sq. ft. attains 27 per cent. control, but the plot receiving 80 gals./acre (with a corrected total expected of 11) still shows a larval increase (64%).

Discussion.

The relative failure of 50 mg. p.p'DDT/sq. ft. to control larvae of *C. impunctatus* in 1955 and the complete failure of half this dosage requires an explanation. Three trials have now been made with the application of p.p'DDT wettable powder at 50 mg./sq. ft.—on 15th December 1953, 10th April 1954 and 29th April 1955. The degrees of residual control attained were 96 per cent., 97 per cent. and 43 per cent., respectively. The season of the year at which the spray is applied appears to be of little importance, because both the December and April applications made in the winter of 1953-54 were highly successful. On the

other hand, the applications in April 1954 and April 1955 differ markedly in their effect, although both trials were conducted with material taken from the same batch of insecticide. It is likely that the cause of this difference will be found in the conditions prevailing in the two years. Before discussing the nature of this factor it will be useful to consider the probable way in which the insecticide treatment operates.

When a spray is applied to a breeding site the insecticide droplets will be retained on the living plants covering the soil. Even an application of 80 gals. per acre is only equivalent to a rainfall of 0.004 in. ($=0.1$ mm.), in meteorological terminology a trace of rain, which will not be sufficient to wash the insecticide on to the peat. Presumably the method relies upon rain to achieve this effect. From the surface the insecticide will slowly be carried in solution and suspension into the peat, either along with drainage water or by diffusion. This would explain the failure of both insecticidal dusts and oil solutions to kill larvae already in the soil, because they do not mix with water. The penetration of wettable powders in suspension will depend upon the pore structure of the soil and they are unlikely to penetrate very far. It was hoped that emulsions formed from water-miscible oils would be carried into the soil more readily, but the extent of this carriage will depend upon the stability of the emulsion. When the emulsion breaks, the preparation will behave more like an oil solution.

For the prevention of reinfestation the insecticide must either kill or repel ovipositing females or kill the newly hatched larvae. According to Hill (1947), the eggs of *C. impunctatus* are laid on the surface of the soil. In order to do this the female must burrow through the dense overlying mossy vegetation, during which time she may encounter insecticidal deposits. From their work on the effect of BHC deposits on females of *C. impunctatus*, Hill & Roberts (1947), concluded that "successful oviposition would, in all probability, be achieved". If this be correct, then the reinfestation is prevented by the insecticidal deposit killing the newly hatched larvae. (From the point of view of control this is more desirable than repelling the ovipositing females.) This larvicidal action will operate during and shortly after the period when the eggs are hatching. Since the incubation period lasts only one to three weeks (Hill, 1947) the critical period for residual control will be similar to that of adult activity, *i.e.*, May to September, inclusive. To produce this result the insecticide must be on the surface or in the superficial layer of the peat. A large volume of rain will be required to wash particulate insecticides such as dusts and wettable powders through the overlying vegetation on to the surface of the peat. Emulsions will be more readily transported as long as they are stable but when the oil phase has separated out the insecticide will be equally difficult to move.

The data available (Table V) can now be considered in the light of this explanation. Between the spraying on 15th December 1953 and the end of April 1954 (pre-oviposition period) 7.37 in. of rain* fell. During the subsequent oviposition period (May–September, inclusive) the rainfall was 17.25 in., making a grand total of 24.62 in. of rain between the application of insecticide and the end of the critical period for reinfestation. This treatment achieved 96 per cent. residual larval control. For the treatment on 10th April 1954 the corresponding figures were:—pre-oviposition rainfall, 0.90 in.; oviposition period rainfall, 17.25 in.; total, 18.15 in., and 97 per cent. residual control was achieved. After the spraying on 29th April 1955 the pre-oviposition rainfall was 0.03 in., the oviposition period rainfall 6.67 in. and the total 6.70 in. The treatment achieved

* Meteorological data, recorded at the Royal Observatory, Edinburgh, were obtained from the Scottish Meteorological Office. They indicate relative rather than actual conditions. According to the rainfall map published by the Ordnance Survey, 1949, Soutra Hill has an annual rainfall of 35 in. and the Royal Observatory of 28 in. Therefore the actual rainfall on Soutra is likely to be about 25 per cent. higher than the figures quoted here.

only 43 per cent. residual control. It would thus appear that 6.70 in. of rain is insufficient for full residual action, while 18.15 in. or more is adequate to distribute the insecticide over the peat in a satisfactory manner.

At a height of 300 ft. above m.s.l. on Loch Lomondside it has been shown that *C. impunctatus* has two periods of abundance during the season—in the first week of June and the third week of July (Kettle, 1950). Soutra Hill, where the present experiments were conducted, is on the same latitude as Loch Lomond but at an elevation of 1,100 ft. a.m.s.l. Under these cooler conditions the seasonal peaks of *C. impunctatus* are likely to occur later in the summer. Therefore the complete failure of the treatments with 25 mg. p,p'DDT/sq. ft.,

TABLE V.

Effect of rainfall on residual action of 50 mg. p,p'DDT/sq. ft.

Date of treatment	Rainfall (in.)			Residual control (%)
	Pre-oviposition	During oviposition	Total	
15.xii.1953 ..	7.37	17.25	24.62	96
10.iv.1954 ..	0.90	17.25	18.15	97
29.iv.1955 ..	0.03	6.67	6.70	43
30.vi.1955 ..	—	3.82	3.82	1

For details see text.

on 30th June 1955, to achieve any residual control cannot be attributed entirely to the late date of application. The dosage is not below the effective level because half this amount (*i.e.*, 12 mg. p,p'DDT/sq. ft.) achieved 80 and 29 per cent. residual control when applied on 15th December 1953 and 10th April 1954, respectively (*i.e.*, 1956, Tables IV & X). It seems clear that the main cause of failure was not dosage or date of application but the low rainfall during the oviposition period. In the three months after the treatments of 30th June 1955 only 3.82 in. of rain fell, compared with 10.43 in. in the same period of the previous year.

If this interpretation is correct, two other points require consideration. Firstly, whether or not the state of the weather immediately after spraying is particularly important. It was observed that the treatment of 10th April 1954 was followed by 19 days during which rain fell only twice (0.012 in. on 16th April and 0.036 in. on 25th April), after which rain fell regularly. During the same 19-day period there was an average of seven hours sunshine a day. Such a period of drought immediately after spraying would leave the insecticide on the vegetation exposed to removal by wind and destruction by sunlight. In fact this treatment produced 97 per cent. residual control, so that its insecticidal activity had not been affected by the dry sunny spell immediately following application.

Conversely, after the spraying on 29th April 1955 it rained every day, except 17th May, until 23rd May, totalling 2.138 in. Similarly, after the treatment of 30th June 1955, 1.539 in. fell in the next three days and yet both these treatments were ineffective. In the first season, however, there was an adequate total rainfall during the later post-spraying and oviposition period, in the second there was not. It is clear, therefore, that the limiting factor is the total amount of rain which falls between spraying and larval emergence from the egg. From this

it follows that insecticidal treatments should be applied as early as possible in order to get maximum effects in the first two seasons.

The second question is, whether or not, once the insecticide has been washed down on to and distributed over the surface of the soil, its residual activity is independent of rainfall during the oviposition period. Independence of such rainfall is indicated by the fact that the 1953 and 1954 treatments remained effective during the very dry summer of 1955 when the current year's treatments more or less failed. The persistence of p,p'DDT in the soil is to be expected in view of the findings of Smith (1948), who records a loss of only 5 per cent. DDT after 18 months in the soil. It should, perhaps, be mentioned that in the Edinburgh area the summer of 1955, and indeed the whole year, was one of the driest on record. Consequently, when the experimental plots were visited on 5th October 1955 it was possible to walk dryshod over ground where normally knee-length rubber boots were essential. The bare areas of exposed peat were covered with a dry, light-brown fluffy deposit in place of the more usual wet slippery black slime.

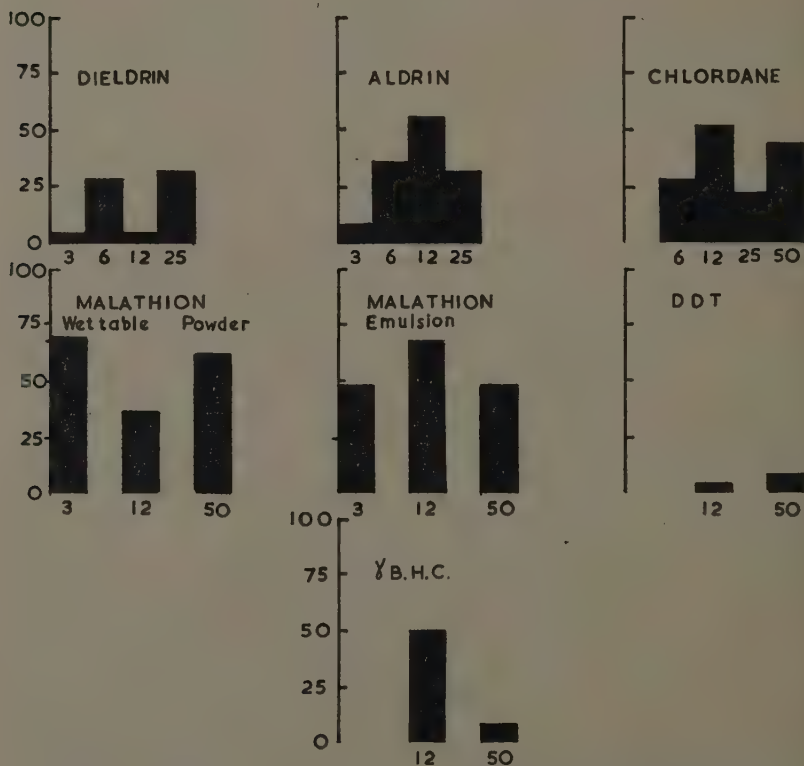


Fig. 1.—Immediate control of larvae of *C. impunctatus* following application of various insecticides at different dosages. Ordinate, percentage larval reduction; abscissa, dosage in mg./sq. ft. Data from Kettle, Nash & Hopkins (1956).

Further support for the view expressed here is supplied by Hill & Roberts (1947), who found that heavy rain increased the penetration and effectiveness of an application of 100 mg. γ BHC/sq. ft. Rain was still necessary even though the volume of spray applied was equivalent to 476 gals./acre. This suggests that, compared with rain, the actual spray volume is unimportant. The essential feature is good initial coverage aided by subsequent prolonged rain. It is noteworthy that the effective residual control of Hill & Roberts (*op. cit.*) and Cameron (1948) were obtained in the summer of 1946, which like 1954 was much wetter than the average.

In an earlier paper (Kettle, Nash & Hopkins, 1956), attention was drawn to the fact that, after the application of insecticides to site 2 in April 1954, the immediate larval mortality was independent of insecticidal dosage. The results concerned are given in the form of a histogram in fig. 1. They suggest that there was a factor, other than dosage, which limited insecticidal effect. In the previous paper it was wondered whether this factor was the constant spray volume in which the insecticides were applied, but from the present discussion it would appear that rainfall is more likely to be the limiting factor. Between the insecticidal applications in April 1954 and the three samplings in May to assess the immediate control, 2.06, 3.41 and 4.05 in. of rain fell. On the scale of rainfall now considered adequate for residual effect of DDT these values are very low, and it is interesting to note that both the DDT dosages achieved no immediate control. The precise relationship between rainfall and insecticidal effect will vary with individual insecticides, depending in part upon their solubility and toxicity. Hence, under similar conditions, malathion achieved about 50 per cent. control, aldrin and chlordane 33 per cent., dieldrin and BHC 20 per cent., and DDT 5 per cent.

Summary.

Further observations and trials were conducted with insecticides against larvae of *Culicoides impunctatus* Goetgh. on Soutra Hill, Midlothian. The analysis of the data was complicated by a large natural reduction (76%) which occurred in the untreated plots. Nevertheless, it appears that the following dosages of insecticidal preparation were still active two years after application:—(a) 50 and 200 mg. p.p'DDT/sq. ft. applied as a dust, wettable powder or water-miscible concentrate (99% control), (b) 50 and 200 mg. γ BHC/sq. ft. as a wettable powder or 200 mg. γ BHC/sq. ft. as a water-miscible concentrate (95% control), (c) dieldrin at 25 mg./sq. ft., which gave 97 per cent. control and (d) chlordane at 50 mg./sq. ft., which gave 84 per cent. control.

The effect of spray volume on insecticidal effect was investigated by applying 25 mg. and 50 mg. p.p'DDT/sq. ft. in the following spray volumes:—5, 10, 15, 25, 50 and 80 gals./acre. The effect of 50 mg./sq. ft. at each volume was less marked than in the previous work, when only one concentration was used. Weather conditions in the present season were unusual, but there were indications that moderate spray volumes (15 and 25 gals./acre) give the best results at this dosage of insecticide. The combined results for 50 mg./sq. ft. at all volumes gave only 43 per cent. residual control, while those for the lower dosage showed no residual control.

The results are discussed with reference to the effect of rainfall on residual control. It is concluded that the volume of spray applied is unimportant provided the droplets of insecticide are closely and evenly distributed on the mossy vegetation that overlies the peat. Subsequent prolonged rain, of the order of 20 inches, is required to attain the most effective distribution of the insecticide, which is at the surface of the peat, where the eggs are laid and the newly hatched larvae will come in contact with the poison.

Acknowledgements.

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THE CUMULATIVE TOXICITY OF DINITRO-O-CRESOL APPLIED IN SMALL DOSES TO LOCUSTS.

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A method of attacking flying swarms of locusts in which the poison is sprayed directly into a swarm by relays of light aircraft flying low over it has been described by Rainey & Sayer (1953). The quantity of insecticide applied in each sortie is usually small in relation to the total amount required to kill the swarm and, although some of the locusts receive a lethal dose immediately, a substantial part of the final mortality is brought about by the accumulation of separate sub-lethal doses picked up by each locust over a period of time.

Successful results have been obtained by this method on a number of occasions, but there was reason to suspect that, especially with the larger swarms, there was some loss in the toxic efficiency of the insecticide when the spraying operation was spread over several days.

The experimental work described below has accordingly been carried out to determine whether, under constant laboratory conditions, sub-lethal doses applied at intervals are wholly additive in their effects; or whether, due to the time factor, a stage is reached at which the insecticide can be eliminated, or otherwise disposed of, by the locust as fast as it is applied.

Few observations have been reported on the cumulative aspect of insecticidal action and none relating specifically in this respect to locusts. Beard (1952), working with four insecticides, found that the interpretation of his results was neither simple nor straightforward, owing to the variable nature of the biological material and the difficulties encountered in distinguishing between the effects of the initial and final treatments. In view of this, it was desirable that in the initial stage of the present experiments the least complicated experimental method should be used. It has been shown that the resistance of a group of locusts to dinitro-o-cresol (DNC) remains constant over a period of at least one month when the dose applied is measured as microgrammes of active constituent per gramme locust weight (MacCuaig, 1956). Accordingly the insecticide used was limited to DNC which, together with its rapid speed of action and steep probit regression slope, could be expected to present the least difficulty in the interpretation of the results. The more direct but rather lengthy method of spraying flying locusts in a wind tunnel (Wootten & Sawyer, 1954) was not attempted, and the technique was restricted to the application of single drops of insecticide.

Method.

The DNC used was in the form of a 20 per cent. solution by weight in a mixture of two aromatic petroleum extracts, known as the Mk. IV solution (Wootten & Sawyer, 1954). Application was by means of a micro-drop syringe, capable of delivering individual drops as small as 2×10^{-5} ml. (coeff. of variation 10%) which could be placed quite accurately (90% within a one-millimetre circle)

on any selected part of the locust. After treatment, the insects were transferred to the relatively constant conditions of 24–27°C. and 60–80 per cent. relative humidity.

Each locust was weighed before dosing and the quantity of insecticide applied adjusted to give a pre-selected amount of active constituent per gramme body weight. In the main series of experiments the insecticide was applied to the intersegmental region between the first and second abdominal sternites, a site of application which has been extensively used for experiments of this kind. A few observations were made with the insecticide applied elsewhere, as detailed later.

The species primarily used was the Desert Locust, *Schistocerca gregaria* (Forsk.), but the experiments were repeated with smaller numbers of the Migratory Locust, *Locusta migratoria migratorioides* (R. & F.).

The insecticide applications were made in two ways; in one, the locust received a regular daily dose, and in the other, two doses only at various time intervals.

In each series, the locusts were divided into three main groups, the largest group being used for the experiments on cumulative effects and the other two for determining the susceptibility of the insects to a single dose and to act as controls. The first two groups were subdivided for dosing purposes. The order in which the locusts were placed in the various sub-groups was predetermined, each insect, when taken from the storage cages, being assigned to a particular sub-group in a strict rotation. This method, although not a true randomisation, is one which is simple to use and which has previously given very satisfactory results.

Results: Dose applied Daily.

Preliminary experiments indicated that an adequate mortality range would be covered by doses ranging from 3 to 9 $\mu\text{g./g.}$ body weight. The actual doses given and the numbers of locusts used are shown in Table I. The increments between the doses were selected so as to be roughly equal on a logarithmic scale. Each locust was dosed at intervals of approximately 24 hours until it died or, if it survived, for a maximum of six days. The nature of the results indicated that this period was sufficient to enable adequate conclusions to be drawn from the experiments.

TABLE I.

Doses and numbers of locusts used.

Daily dosing										
Dose ($\mu\text{g./g.}$)	0	3.3	4.2	5.25	7.0	9.4	
Numbers of <i>S. gregaria</i>	69	44	56	60	68	58	
Numbers of <i>L. migratoria</i>	25	—	20	18	18	20	
Single doses										
Dose ($\mu\text{g./g.}$)	8.0	9.4	10.5	12.0	14	15	18
Numbers of <i>S. gregaria</i>	20	58	30	42	18	29	31
									10	
Dose ($\mu\text{g./g.}$)	7.0		9.0	13.6		16.0	
Numbers of <i>L. migratoria</i>	18		20	15		15	

General statement.

The percentage daily kills with *S. gregaria* are shown in fig. 1. It is apparent that substantial non-cumulative effects occurred, since virtually all the kills were obtained by doses applied during the first four days, the deaths during this period

being 101 (35% of the total number of insects used) while the fifth and sixth doses only added another 12 (4%).

With *L. migratoria* (fig. 2) there was only one death from the fifth dose (the experiment then being discontinued) as against 55 from the first four doses.

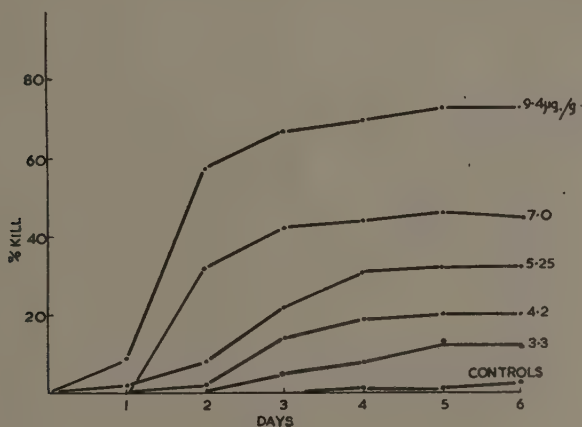


Fig. 1.—Kills obtained with daily dosing (*S. gregaria*).

Ratio of single to daily dose.

Using as the final result the 4-day kill, the regression equations obtained with *S. gregaria* and *L. migratoria* are shown in Table II and plotted in figs. 3 and 4, respectively.

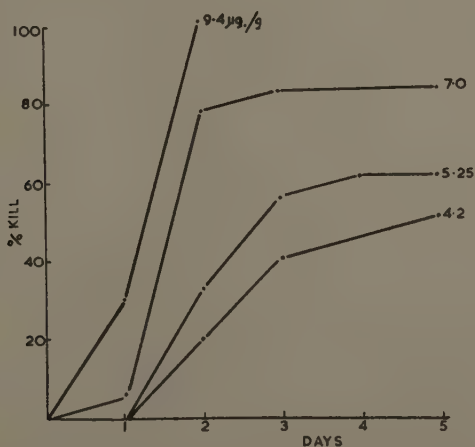


Fig. 2.—Kills obtained with daily dosing (*L. migratoria*).

Since the slopes of the regression equations in fig. 3 are different, it is impossible to derive a single overall factor to compare the effects of daily doses with a single dose. The daily dose required to achieve a 50 per cent. kill in four days was $7.2 \mu\text{g./g.}$, i.e., a total of $29 \mu\text{g./g.}$, as compared with $12.7 \mu\text{g./g.}$ to give the same kill with a single dose. In terms of total quantity of insecticide applied, the daily dosing is thus less than half as effective as the single dose.

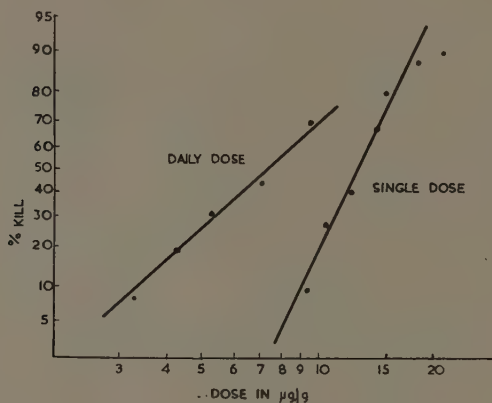


Fig. 3.—Probit regression lines for single and daily doses (*S. gregaria*).

The efficiency of the daily dose decreases as the kill rises and if the data could be linearly extrapolated to a 90 per cent. final kill, the total of the daily doses required would be approximately three times the single dose.

With *L. migratoria* the two lines are very nearly parallel, as shown in fig. 4. The total daily dose required to give a 50 per cent. kill is approximately 1.5 times the single dose and the ratio does not alter appreciably at other mortalities.

Relative susceptibilities of the sexes.

The results given in Table II relating to *S. gregaria* were analysed separately for each sex as shown in Table III.

TABLE II.

Regression equations, single and daily doses.

		Regression equation	Standard error of slope	LD50 and standard error	χ^2
<i>S. gregaria</i>					
Single dose	..	$y = 8.94x - 4.88$	0.98	12.73 ± 0.33	4.0 (4)
Daily dose	..	$y = 3.98x + 1.58$	0.57	7.23 ± 0.37	0.8 (3)
<i>L. migratoria</i>					
Single dose	..	$y = 5.63x - 1.11$	1.37	12.2 ± 0.87	0.35 (1)
Daily dose	..	$y = 5.82x + 1.22$	1.48	4.47 ± 0.51	1.53 (1)

The analysis suggests that in these experiments the females were slightly more resistant to a single dose than the males (relative susceptibility, males to females = 1.09), but since the confidence limits are 0.99–1.20 the data are barely sufficient to establish the point. However, when the doses are applied daily, the quantities required to give a 50 per cent. kill in four days are in the ratio of 1.54:1 (limits 1.23–1.94) which indicates a significant difference in the susceptibility of the

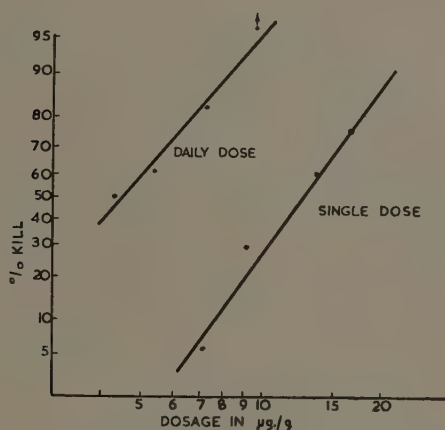


Fig. 4.—Probit regression lines for single and daily doses (*L. migratoria*).

sexes. Taking into account the different weights of the sexes (see p. 441), it follows that the females require more than twice as much insecticide per locust to obtain the same percentage kill of both sexes.

At higher mortality levels, the relative susceptibility appears to decrease slowly, but the slopes of the lines are not significantly different and extrapolation beyond the range of the experimental mortalities obtained is of doubtful validity.

TABLE III.

Regression equations, daily and single doses: males and females (*S. gregaria*) compared.

	Regression equation	Standard deviation of slope	LD50 and 95% confidence limits (µg./g.)
Single dose			
Males	$y = 8.46x - 4.03$	1.39	11.7 10.9—12.5
Females	$y = 9.22x - 5.20$	1.32	12.8 12.0—13.6
Daily dose			
Males	$y = 4.04x + 1.98$	0.81	5.58 4.81—6.40
Females	$y = 5.07x + 0.26$	0.97	8.61 7.57—10.6

With *L. migratoria*, the results in aggregate showed a similar trend, but the numbers of insects used were too few to enable a detailed analysis to be carried out. Thus the number of males killed with a single dose was 49 per cent., compared with 48 per cent. of the females. With daily doses, the proportion of males dying (82%) was considerably greater than that of females (47%), giving a susceptibility ratio of approximately 1.5.

Effect of temperature.

From the results of Potter & Gillham (1946) relating to the effects of post-treatment temperature on mortality, it might be expected that, in locusts surviving the first daily dose, the processes causing a loss of toxicity would be accelerated at higher temperatures. A short experiment to test for this effect was carried out at two temperatures.

Two groups of 16 locusts each, taken from a batch shown to be homogeneous by a subsidiary experiment, were dosed with 10 µg./g. on two successive days. One group was kept at the usual holding temperature, about 26°C., and the other at about 37°C. The kills obtained were identical, 88 per cent. after the second dose at each temperature.

Successive doses applied to different parts of the body.

A further small experiment was carried out with *S. gregaria* to ascertain the effect of applying the dose to different parts of the insect on successive days. The parts chosen were all known from previous experiments to be approximately equally susceptible. In order of dosing, they were:—

- (1) the ventral surface of the abdomen (the standard position);
- (2) the trophi, the drop being aimed between the jaws;
- (3) the dorsal surface of the base of the right hind wing;
- (4) the dorsal surface of the abdomen; and
- (5) the junction between head and pronotum.

The results obtained were very similar to those of the previous experiments. The total number of locusts dosed was 61, and of 31 that died during the five days of the experiment, only two did so as a result of the fifth dose. The regression equation for a single dose and for four daily doses gave the ratios of LD50 single to daily doses of 1.91, as compared with 1.74 obtained from the general results.

TABLE IV.

Estimated LD50 values when the dose is applied in two equal parts with a varying interval between the applications.

S. gregaria

Interval (hours)	0	24	48	72
LD50 (µg./g.) Experiment 1	12.5	15.0	16.8	22.3
2	12.0	15.0		
3	11.5	12.7		
4	13.4	18.0	23.0	

L. migratoria

Interval (hours)	0	7	16	24	30	72
LD50 (µg./g.) Experiment 1	13.3	11.2	13.2		18.2	24.5
2	12.2			11.2		

Effect on the weight of the locust.

The mean weight of the controls (females 3.23 g., males 2.18 g.) remained nearly constant throughout the experiments, but the weight of the dosed individuals fell sharply between the first and second applications (females 3.24 to 3.09 g., males 2.13 to 2.02 g.). From the second to the fourth day (and, where measured, the fifth), the weights changed very little. The daily loss of weight in a group of starved locusts was almost the same as that of the dosed locusts during this period.

Results: Dose applied in two Applications at various Time Intervals.

From the experiments described above, it was possible to determine, with the aid of a few supplementary experiments, the mortality of *S. gregaria* obtained from equal doses of DNC applied at time intervals varying from 0 to 72 hours. For a given interval, the final kill obtained plotted against the total dose applied gave the median lethal doses shown in Table IV.

A separate experiment was carried out on a single batch of some 200 individuals of *L. migratoria* divided into five approximately equal groups, one each for time intervals of 0, 7, 16, 30 and 72 hours. The probit dosage regression line for each time group had about the same slope as that of the single-dose group. Using this slope, the LD₅₀ values shown in Table IV were obtained graphically. Additional points obtained from the daily dosing results are included.

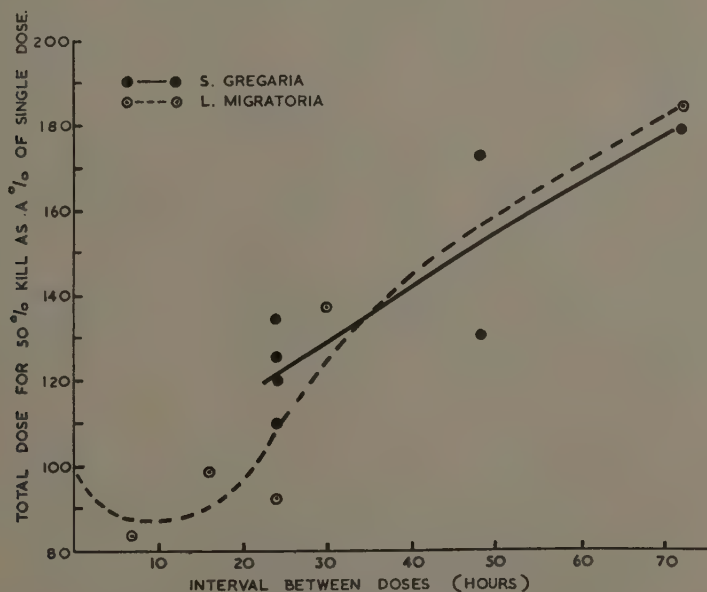


Fig. 5.—Susceptibility to two doses of DNC with a varying interval of time between them.

The results are conveniently expressed diagrammatically in fig. 5 where the split dose is plotted as a percentage of the single dose required to yield the same kill. A value below 100 per cent. represents sensitisation and 200 per cent. complete loss of effectiveness in the interval.

Discussion.

(1) The two types of experiment, dose applied daily and dose applied in two parts, are in excellent agreement. From the daily-dose results, the fact that deaths after the first four days are very few indicates that the insecticide applied on the first day must, by the fourth, have been almost completely eliminated or detoxified by the insect. Similarly, from the second set of experiments, when the dose is applied in two instalments, it can be deduced that about 80 per cent. of the first must have been rendered ineffective after three days.

(2) Since the probit dosage relationships can be represented by straight lines within the range of mortalities determined, the ability to cope with a particular daily dose appears to be distributed in much the same manner as resistance to a single dose. The dose retained by each insect is a function of its rate of elimination as well as the rate at which the poison is applied, and the insect dies when the retained dose reaches the value which it is unable to withstand. With some individuals, this never happens because the accumulating dose does not exceed the tolerance of the individual concerned.

To explore further how far the ability to tolerate a daily dose is correlated with the ability to resist a single large dose, some of the survivors from the daily dosing experiments were given an additional dose after an interval of five days. It was estimated that in this period substantially all the accumulated effects of the earlier application should have been eliminated. Each locust was given 2.2 times the daily dose it had formerly received. The mortality expected from this treatment, had they been a normal population, was 30/80; the kill actually obtained, 7/80, was significantly lower. If the correlation between the resistance to the two methods of dosing had been complete, then all the insects would have been expected, on the basis of the preceding experiment, to survive a single dose 2.5 times the daily dose. The small kill obtained, 7/80, from a dose of only 2.2 times the daily dose, thus suggests that the correlation between the susceptibilities to daily and single doses is not quite complete, but the possibility that the interval of five days may not have been sufficient to eliminate completely the effects of the previous dosing cannot be ruled out. The fact that the slopes of the daily- and single-dose regression lines for *S. gregaria* in fig. 3 are significantly different ($t = 4.35$, $P < 0.01$) also suggests that the correlation between the two susceptibilities is not complete.

(3) There is no significant difference in susceptibility between the species when the poison is applied as a single dose, but, when the dose is given daily, *L. migratoria* becomes the more susceptible and the ratio of the median lethal doses between the species rises to 1.45 (95% confidence limits 1.1-1.9) at the end of

TABLE V.

Relative susceptibilities of males and females.

	LD50 ($\mu\text{g./g.}$): dose applied in two halves with approximately 24 hours' interval		Daily dose giving 50% kill in 4 days	
	<i>S. gregaria</i>	<i>L. migratoria</i>	<i>S. gregaria</i>	<i>L. migratoria</i>
Males	14.7	13.3	5.6	ca.3.8
Females	21	13.7	8.6	ca.5.8
Relative susceptibility, males/females	1.42	1.03	1.54	1.5

four days. When the dose is applied in two halves, it appears from fig. 5 that with *L. migratoria* the doses are at least fully cumulative and there may be some degree of sensitisation during the first 24 hours. After that time, the decreasing effectiveness of subsequent doses appears to be similar to that in *S. gregaria*. Corresponding initial effects with *S. gregaria* were not detected as the minimum dosing interval was 24 hours, but the form of the curve in fig. 5 suggests that they would have been much smaller than in *L. migratoria* or even absent altogether.

(4) Another interesting aspect of the results and one which is of considerable practical significance is the difference in the susceptibility of males and females to daily dosing.

To a single dose, males and females are almost equally resistant, and there is no difference in this respect between the species. When the dose is applied in two halves over 24 hours, there is still no difference in *L. migratoria*, but a significant difference between the susceptibilities of the two sexes appears in *S. gregaria*. When the dosing is spread over four days, a sex difference occurs also in *L. migratoria* and the final relative susceptibilities in both species appear to be very nearly the same, as shown in Table V.

(5) For practical purposes the results with *S. gregaria* can best be summarised in the form of fig. 6, which shows how the effectiveness of a given quantity of insecticide decreases when the application, in equal instalments, is spread over a period of time. If, as may reasonably be assumed, similar non-cumulative effects occur with flying locusts, it is evident from fig. 6 that serious losses in

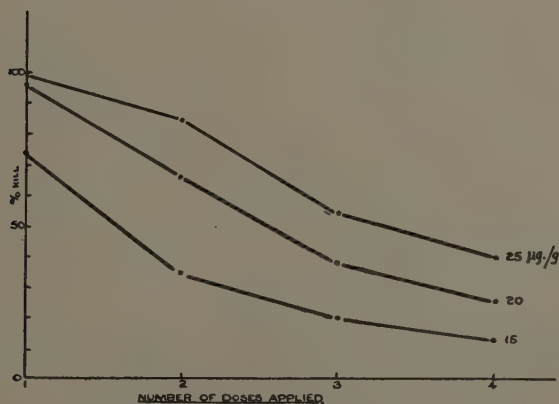


Fig. 6.—Loss in effectiveness of a given quantity of DNC when application in equal doses at 24-hr. intervals is spread over a period of time (*S. gregaria*).

efficiency could occur in an air-spray operation if this were unduly protracted. If the operation were spread over four days, for example, it is evident that the kill obtained from a dose of 25 $\mu\text{g./g}$. of swarm would be about 40 per cent., whereas if it had been possible to apply the whole dose evenly over the swarm in one day the kill would have exceeded 99 per cent. Moreover, because of differences in susceptibility between the sexes, the 60 per cent. surviving would be

predominantly female and would represent more than two-thirds of the original egg-laying capacity of the swarm.

(6) By the method of experiment used, there was little opportunity for the poison to be removed by contact with extraneous objects, so that the loss of effectiveness of the insecticide with time must have been almost entirely due to physiological causes. For practical purposes this represents a minimum loss in effectiveness; when the dose is distributed over a considerable time interval in the field, the loss may be rendered greater than that shown in fig. 6 by, for example, contact losses on roosting and evaporation of DNC during flight.

Summary.

By the method of attacking flying swarms of locusts with insecticides sprayed directly into the swarm by relays of light aircraft, a proportion of the swarm receives a sub-lethal dose from each sortie. For successful operation it is essential that a substantial contribution to the final mortality should be produced by the accumulation of these sub-lethal doses on individual insects over a period of time. The object of the present experiments was to determine whether such doses applied at intervals are wholly additive in their effects. The poison used was dinitro-o-cresol (Mk. IV DNC solution) and to shorten the experimental procedure, the locusts, *Schistocerca gregaria* (Forsk.) and *Locusta migratoria migratorioides* (R. & F.), were dosed by means of a single drop of poison applied to the ventral surface of the abdomen by a micro-drop syringe.

When locusts are given regular daily doses of DNC the doses are not wholly cumulative in their effect. After the second or third day the lethal effect of each dose becomes steadily less, and after the fourth or fifth day it tends to zero, representing a steady state in which the rates of application and loss of insecticidal activity in the survivors are equal.

When the dose is applied in two halves with various time intervals between them, the cumulative effect during the first 24 hours is less in *S. gregaria* than in *L. migratoria*. In the latter species it is possible that sensitisation occurs. After three days, the first half-dose has fallen to an estimated 20 per cent. of its initial effectiveness in each species.

These two species are equally susceptible to a single dose expressed as $\mu\text{g. DNC/g. body weight}$. The females are more resistant than the males to daily doses (relative susceptibility in *S. gregaria*, 1.54) but probably not to single doses (relative susceptibility, 1.09).

Resistance to a daily dose correlates roughly with resistance to a single dose, the total dose required to produce 50 per cent. mortality in four days being about twice the LD50 for a single dose.

If flight activity does not materially alter the present results, it is evident that the non-cumulative effects of sub-lethal doses could cause a serious loss in the efficiency of an air-spray operation if it were unduly prolonged. Thus a quantity of insecticide sufficient to kill over 99 per cent. of the locusts if applied as a single dose would kill less than 40 per cent. if the application were spread over four days.

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STUDIES ON WHEAT BULB FLY (*LEPTOHYLEMYIA COARCTATA* (FALL.)).

IV.—THE DISTRIBUTION OF DAMAGE IN ENGLAND AND WALES IN 1953.

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The serious attacks of Wheat Bulb Fly, *Leptohylemyia coarctata* (Fall.), in 1953 offered an opportunity for officers of the National Agricultural Advisory Service to collect information on the amount and distribution of the damage caused by this pest. This paper is an attempt to collate and analyse some of the material critically and summarise the observations.

In a general account of the fly (Gough, 1953), it was suggested that the reason for the serious damage in 1953 was the coincidence of two major factors: (1) fly populations were higher in the summer of 1952 than they had been for some years and weather conditions at the time of egg-laying were probably very favourable. As a result, many more eggs than usual were laid that summer. (2) The relatively low temperatures of the autumn of 1952 held back the germination and development of the wheat crop so that it was mainly in the single-shoot stage when the eggs hatched in January and February 1953 and was therefore very susceptible to damage. M. J. Way (*in litt.*) has since pointed out that the effect of the low autumn temperatures on the egg in diapause would probably result in earlier hatching.

The evidence for these suggestions is briefly given here. The intensity of attack in the early part of 1952 was the heaviest since 1945 and it seems reason-

TABLE I.

Wheat Bulb Fly egg numbers per acre in fenland soils.

Year	No. of fields	Geometric mean (in thousands)
1952	12	1630
1953	46	420
1954	31	410
1955	26	358

able to suppose that the number of flies emerging in the summer of 1952 was greater than for many years. Although little is known about the effect of weather conditions on the flies, by analogy with other Anthomyiids it is probable that the dry June and July of 1952 were very favourable for the maturation and oviposition periods.

In 1952, the first of a series of egg counts was made on fenland soils (mainly peats, but including a few silts and gravels). Subsequent counts in 1953-55 have

confirmed (see Table I) that the egg numbers were high in 1952. Most of the counts were made on potato fields but a few crops of sugar beet and vining peas were also included. The egg counts were based on twenty 4-inch cores in each field taken to the depth of cultivation, the sample being subsequently examined by a flotation method. The results, expressed as the geometric mean of the number of eggs per acre in each year, are given in Table I.

Although only a few fields were examined in 1952, the numbers were remarkably uniform and the lowest count was 975,000 per acre whereas in later years the majority of fields had fewer than 500,000 per acre. The level of attack in the years following those with lower egg numbers was comparable to that in the period 1946-51.

The departures of temperatures from the monthly mean in eastern England in the period between egg-laying and hatching in 1952-53 were as follows:—

Month					°F.
September	-4.2
October	-1.7
November	-3.5
December	-3.0
January	-1.6

Thus, during the whole period likely to affect the establishment of winter wheat, temperatures were appreciably below normal.

The result of all these factors was that in areas where Wheat Bulb Fly is normally a pest, and in adjoining areas where usually only occasional failures occur, there was widespread and serious damage, and in districts where larval populations are normally so low that they are scarcely noticeable, the population increase was often sufficiently great to have a marked effect on the crop.

Total Amount of Damage.

Advisory Entomologists were asked to report the acreage of wheat affected in varying degree and their information in turn was often based on the reports of District Advisory Officers in the various counties. In the areas with little damage, the figures given were usually the total acreage of fields where the cause of damage was confirmed. For Yorkshire and Lancashire, East Midland and Eastern Provinces the figures were usually carefully considered estimates based on the number of attacked wheat fields visited by the N.A.A.S. District Officer. In one county of the Eastern Province (Isle of Ely) a questionnaire was sent to selected farmers whose land in total represented about 1/7th of the county acreage and who were known to keep careful records. The acreage they recorded for each damage category (see below) was then used to calculate the approximate total damage for the county.

Figures of this sort are always subject to large errors but these are the best available and it is probable that the totals are of the right order.

The acreage was recorded in three classes chosen as far as possible objectively according to the action taken by the farmer:

- (1) Complete failure—where the farmer decided to re-drill.
- (2) Partial failure—where the farmer decided to patch.
- (3) Affected—where the infestation was noticeable but where the farmer took no action.

In some fields in categories (1) and (2) the farmer's action may have been unnecessary, but equally in category (3) action was not always taken where it would have been desirable.

The figures for the eight Provinces (Scott Watson, 1946, map p. 379) of the National Agricultural Advisory Service are given in Table II.

It is always difficult to convert estimates of damage into monetary values but

it is important to attempt it and to this end the following assumptions have been made:

- (1) The cost of seed wheat was £3 per cwt.
- (2) Complete failures were re-drilled with spring wheat at 4 bushels per acre.
- (3) Partial failures were patched with spring wheat at 2 bushels per acre.
- (4) Spring wheat would yield about 7 cwt./acre less than winter wheat. This difference is based on the mean yields of spring and winter wheat variety trials in Eastern Province and throughout the country in 1953.
- (5) The value of wheat harvested in 1953 was 30/- per cwt.

TABLE II.

Acreage of wheat affected in N.A.A.S. Provinces in 1953.

Province	Total wheat acreage per Province	Acreage failed	Acreage patched	Acreage otherwise affected
Wales	35,035	0	0	0
Northern	55,167	15	10	27
South-West ..	182,182	32	72	5
South-East ..	332,350	350	48	392
West Midland ..	237,877	200	2,000	?
Yorks. & Lancs. ..	241,535	3,614	5,924	15,185
East Midland ..	415,614	23,000	24,000	33,000
Eastern	642,906	32,000	26,000	36,600
Total	2,142,666	59,211	58,054	85,209

Some of the failures were re-drilled to spring barley or other crops and though in some cases these may have been more profitable than wheat it is improbable that their total value would be greater than that of spring wheat.

The minimum financial loss is calculated as follows:—

Cost of new seed at 4 bushels/acre on 59,000 acres	£398,250
Value of reduced yield of spring wheat as compared with winter wheat on 59,000 acres	£619,500
Cost of new seed at 2 bushels/acre on 58,000 acres	£195,750
Total	£1,213,500

If the acreage figures and the assumptions are substantially correct, the minimum financial loss caused by Wheat Bulb Fly in 1953 was about £1,200,000. To this may be added other losses impossible to estimate:

(1) Probable reduced yield on the patched area of 58,000 acres not only due directly to the pest but also to inconvenience, delays and losses through harvesting a mixed crop.

(2) Additional cost of cultivations and fertilisers for spring sowing and general inconvenience and delays in sowing other crops.

(3) Possible reduced yield on some of the 85,000 "affected" acres.

It is interesting to compare the figure of 3,614 acres failed and 5,924 acres patched in Yorkshire (there were no serious attacks in Lancashire) with the acreage in that county which has been estimated (Gough, 1949) as subject to attack on the basis of survey work. It was suggested then that the upper limit for the acreage in Yorkshire where attack by Wheat Bulb Fly was likely to be serious would lie between 7,000 and 10,000. On the basis of experience at that time it was thought that crop loss was only likely on two or three thousand acres in an unfavourable season.

Effect on National Production.

The total wheat acreage for England and Wales in 1953 was 2,142,666 acres* compared with 1,962,850 acres in 1952. The only English county which did not record an increase was the Isle of Ely where there was a decrease of nearly 10 per cent., and it is significant that this was the county with the greatest damage by Wheat Bulb Fly, 30 per cent. of the acreage being re-drilled.

Although the acreage estimated as being seriously affected is only about 5 per cent. of the total wheat crop it might be expected that yields in general for 1953, at all events on the eastern side of the country, would be rather lower than usual. In fact, the mean yield for England and Wales for 1953 was 24.0 cwt. compared with the 10-year average (1944-53) of 20.5 cwt., and an increase on its 10-year average was recorded for each county. Despite the apparently unfavourable winter, 1952-53 was therefore a very good season for wheat.

In order to see if the county average yields reflected damage by Wheat Bulb Fly, the recorded yields of selected counties were inspected and compared for years of heavy and light attacks. The degree of attack detailed below is based largely on my own experience in Yorkshire up to 1947 and subsequently in East Anglia:

- | | |
|----------|---|
| 1943. | Generally light attacks with a few severe ones. |
| 1944. | Numerous severe attacks. |
| 1945. | " " " |
| 1946. | Generally light attacks with a few moderate ones. |
| 1947-50. | Pest relatively unimportant and my personal experience of it limited. |
| 1951. | Generally light with a few severe attacks. |
| 1952. | Numerous severe attacks. |
| 1953. | Exceptionally numerous and widespread severe attacks. |

The five counties (Group A) most severely affected in 1953—Isle of Ely, Kesteven (Lincs.), Notts., Lindsey (Lincs.) and Hunts., were compared with five counties (Group B) with moderate attacks in that year—Holland (Lincs.), W. Suffolk, Essex, Beds. and Cambs., and five counties (Group C) where little or no attack was reported but where large acreages of wheat were grown—Salop, Wilts., Hants., Staffs. and Glos. The yields for the years 1943-1946 were compared, taking 1943 as 100, and the yields for the years 1951-1953 were based on 1951 as 100. The means of each series of five counties are shown in Table III.

The lower yield for group A for 1944 and 1945, years of severe attack, and the relatively greater increase in 1953 for groups C and B as compared with Group A suggest a possible correlation with damage by Wheat Bulb Fly. It is perhaps also worth noting that, of the counties growing over 20,000 acres of wheat, the only ones which did not show an increase in 1953 over 1952 were the Isle of Ely, Holland (Lincs.) and Lindsey (Lincs.). On the other hand, increases were recorded for Kesteven (Lincs.) and Nottinghamshire, which were also severely affected by Wheat Bulb Fly.

* These figures are based on the June returns, i.e., the acreage of winter and spring wheat then in the field and likely to be harvested.

All these figures suggest that there is a possibility that damage by Wheat Bulb Fly in years of severe attack may result in a loss in yield in certain counties of the order of 5 per cent., but too much weight should not be attached to this suggestion as the yield figures themselves are based on parish estimates which must be to some extent subjective. Even if the suggested trends could be proved to be statistically significant there might be many other explanations, as seasonal

TABLE III.

Relative mean yields of wheat for groups of counties in different years.

County Group	Damage 1953	Period 1943-46				Period 1951-53		
		1943	1944	1945	1946	1951	1952	1953
A	severe	100	92	95	99	100	104	107
B	moderate	100	102	97	97	100	103	110
C	none	100	98	100	91	100	104	113

factors affecting the distribution of the pest probably also influence the growth of wheat. It should also be noted that the damage in 1952, when there was no evidence of a difference in yield between the three county groups, was of the same order as that in 1944 and 1945 when a difference was apparent. Thus, although Wheat Bulb Fly is probably the most important insect pest of wheat in England, its effect on the national yield is not likely to be so great as purely seasonal factors; nevertheless within certain limited but widely scattered localities it can be a definite menace.

Distribution in England and Wales.

It is immediately apparent from Table II that the Eastern and East Midland Provinces are the ones most seriously affected, with a smaller but still appreciable acreage in the Yorkshire and Lancashire Province. A preliminary mapping of the distribution on a county basis suggested that this unit was inconveniently large and finally the N.A.A.S. "district" was chosen. These districts vary in size according to the type of agriculture but in general range from 40,000 to 100,000 acres of arable crops and grass (excluding rough grazing). The smaller counties may have only three or four districts but the larger ones may have 12 or more. It was usually possible to obtain reports of the extent of damage in individual districts, and, moreover, cropping figures were also readily available. The more westerly and some of the northern areas of England and the whole of Wales where the wheat acreage is low and where the pest had not been reported have been mapped on a county basis.

The reports from each district were classified into five categories. For most of the counties where serious damage occurred, acreage figures were available, but for the remainder, the District Officer's report was interpreted into the most appropriate category. In order to be as objective as possible, districts were treated as a whole even though part may have been badly affected and part little affected. The limits for categories 3, 4 and 5 refer to the combined acreage of re-drilled and patched crops (serious attacks) expressed as a percentage of the total wheat acreage for the district.

The five categories are as follows:

(1) No reports of Wheat Bulb Fly.

- (2) Wheat Bulb Fly noted as present but no serious attacks reported.
 - (3) Light damage—0·1–1 per cent. of the wheat acreage seriously attacked.
 - (4) Moderate damage—1–10 per cent. of the wheat acreage seriously attacked.
 - (5) Severe damage—over 10 per cent. of the wheat acreage seriously attacked.
- The distribution mapped in this way is shown in fig. 1. It is very similar to that given by Thomas (1948) though he only used three categories and did not

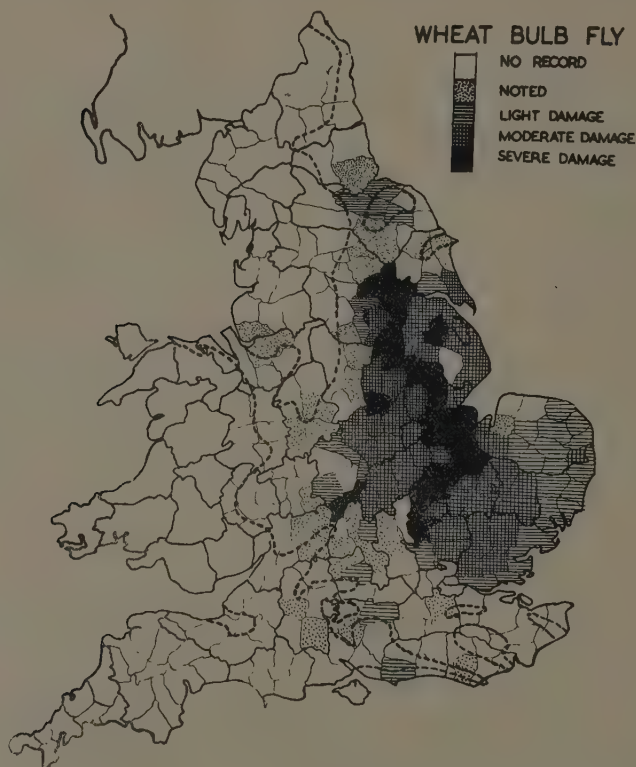


Fig. 1.—Distribution of damage by Wheat Bulb Fly in N.A.A.S. districts in 1953. The dotted line is the isohyet for an annual rainfall of 30 in., the eastern side of the country having less than that.

distinguish between the grades of more serious damage. It is important to emphasise that the map shows the distribution of Wheat Bulb Fly as a pest of wheat and there is therefore bound to be some association with the distribution of wheat.

Acreage statistics for wheat are not available for winter and spring sowings separately but by far the greater proportion is winter wheat and this proportion does not vary greatly from one part of the country to another.

The incidence of Wheat Bulb Fly must depend partly on the proximity of a wheat field to a field which was in wheat the previous year, and this proximity

will be related to the proportion of the total acreage occupied by wheat in a given locality. It was thought that the total acreage of arable crops and permanent grass (excluding rough grazings) would be the most suitable standard for comparison and this is referred to henceforth as "total crops and grass".

A preliminary plotting of percentage attack against wheat acreage expressed as percentage of the total crops and grass for each district, suggested that there was little damage where the wheat acreage was less than 10–12 per cent. Above that figure, although there was a tendency for the attack to increase with increasing wheat acreage, there was considerable variation. This is only to be expected as the likelihood of attack must depend to a great extent on the crops which wheat commonly follows in a given district. Unfortunately there are no figures for crops which precede wheat, but districts where the wheat acreage is high are usually intensive arable areas where the root acreage is also high, and the probability of wheat following a root crop is greatly enhanced. The distribution of the districts where the wheat acreage is over 12 per cent. and over 18 per cent. of total crops and grass is shown in fig. 2 and it can be seen that there is some general agreement with distribution of Wheat Bulb Fly.

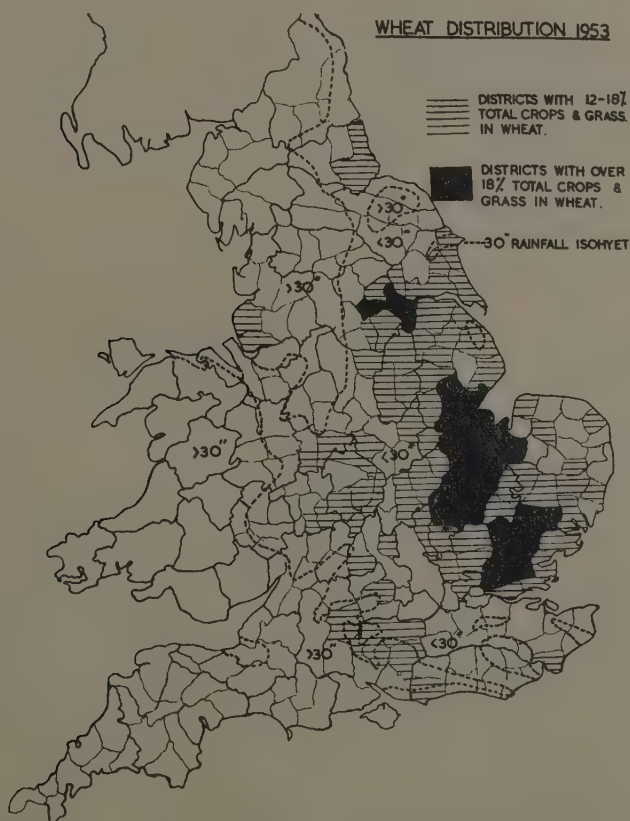


Fig. 2.—Distribution of wheat in N.A.A.S. districts in 1953.

It has been previously pointed out (Gough, 1949, 1953) that attacks by Wheat Bulb Fly tend to fall into two types. On light land, damage most frequently occurs on wheat after potatoes, whereas on heavy land it usually occurs on wheat after fallow. These facts must be taken into account in attempting to correlate distribution of Wheat Bulb Fly with cropping.

As large acreages of potatoes are not generally grown on heavy land, the heavy-land type of attack can be more or less eliminated from consideration of the light-land type by rejecting districts with less than 5 per cent. of potatoes. If the percentage attack for the remaining districts is then plotted against the percentage of potatoes, some relationship between the two is suggested as shown in fig. 3.

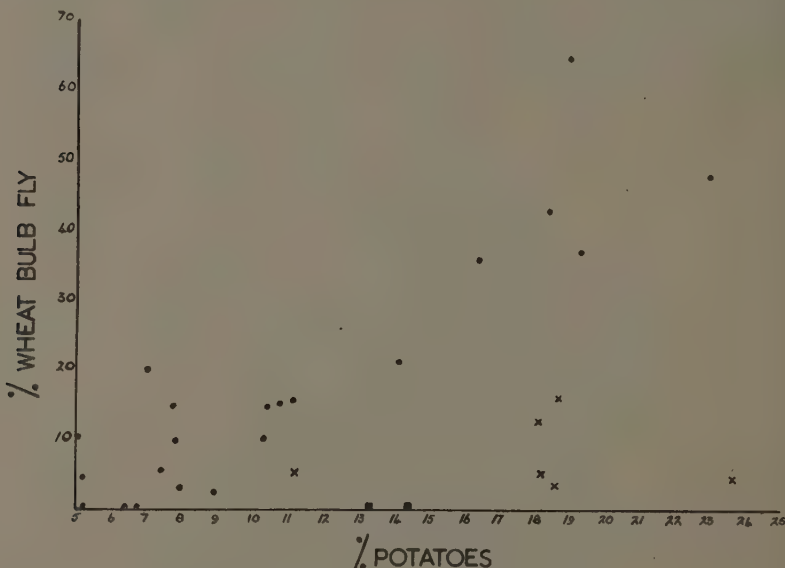


Fig. 3.—Relationship of percentage of wheat attacked by Wheat Bulb Fly to percentage of land cropped with potatoes in N.A.A.S. districts in 1953. For explanation of symbols, see text.

This indicates that, for potato-growing areas, the higher the proportion of potatoes the greater is the probability of serious damage by Wheat Bulb Fly. The group of districts indicated by a cross are all adjoining and mainly silt land areas in Holland (Lines.) and Norfolk. Their divergence from the main trend line is probably a reflection of different cropping sequences and perhaps the high level of soil fertility, but some direct effect of soil or climate on the pest itself cannot be excluded. It is worth pointing out that the map suggests that the heaviest damage has occurred inland rather than near the coast. The two districts indicated by squares, in fig. 3 (13–15% potatoes), are the two on either side of the Mersey estuary, and here it would seem that, although cropping conditions which favour Wheat Bulb Fly on the eastern side of the country exist there, the fly, although it is present, has not developed as a serious pest.

In heavy-land areas, attacks by Wheat Bulb Fly occur after bare fallows (land not carrying a crop but regularly ploughed or cultivated between March and

September) and bastard or half fallows (land cropped, usually with grass or forage crops, in the early part of the year, and ploughed in June or July). Acreage figures are available for bare, but not for bastard fallows, and though there is a tendency for both to occur in the same areas, the proportion of one to the other will vary for different districts. Thus, although areas with a relatively high proportion of fallow give some indication of where the heavy-land type of attack is likely to occur, existing information is insufficient to attempt to show a relationship as has been done for potatoes.

It does seem, however, that much of the distribution of Wheat Bulb Fly as a pest in this country can be explained on a basis of cropping and rotations. Schnauer (1929) could not find a relationship between distribution of damage and areas in Germany where a high proportion of rye or wheat was grown. He showed that the southern limits of the fly on the continental mainland agreed closely with a line bounding the zone having less than $5\frac{1}{2}$ months with a day temperature of 10°C . and over. Bremer (1931), however, pointed out that these limits did not apply in western Europe and they clearly do not hold for Great Britain. Many authors, e.g., Becker & Blunck (1927), Crüger & Körting (1931) thought that moist areas and seasons were unfavourable for the fly, and Rostrup (1924) and Kleine (1918) pointed out that attacks were less in the wetter areas of a field. It has also been noted (Gough, 1947) that very few eggs were found in a potato field with a rather wet soil only a short distance from a potato field with a drier soil in which oviposition was heavy.

Thomas (1948) showed a close parallel between the isohyet for an annual rainfall of 30 in. and the distribution of Wheat Bulb Fly as a pest in England and Wales. This isohyet has been superimposed on figs. 1 and 2, but as it also coincides closely with the wheat-growing area and little information is available about the ecology and preferences of the fly, it seems wiser to reserve judgment on the relationship.

Tentatively, however, one may suggest on circumstantial evidence that the drier eastern side of England is more favourable to the fly than the western, quite apart from the fact that the eastern grows more wheat. On the eastern side of the country it is likely that wheat distribution and the type of crop after which wheat is taken can account for much of the observed variation in the distribution of Wheat Bulb Fly. For example, the lower incidence of Wheat Bulb Fly in south-east England (fig. 1) corresponds with an area where little wheat is grown (fig. 2). This also applies to E. Norfolk, though another factor here would be that wheat is frequently taken after a ley rather than after potatoes or fallow.

It would be interesting to have detailed information about the distribution of the fly and its breeding habits in areas where wheat is not grown to any great extent. It is well known that it can live on many species of grasses (Gough, 1946; Stokes, 1955). Miles & Miles (1955) state that it is common along hedgerows and dykes in East Kent and Romney Marsh.

Correspondence with a few Dipterists who have collected on the western side of the country does not suggest that the fly is common there. Mr. E. A. Fonseca (*in litt.*) has found it at Freshford in Somerset and Mr. G. H. Wallace Pugh (*in litt.*) has found it locally common on the banks of the River Vyrnwy on the Welsh Border on flat alluvial land subject to flooding and some distance from any wheat. All other records supplied by these and other Dipterists were in areas of the country where Wheat Bulb Fly is known as a pest.

Distribution in Scotland and Ireland.

For the sake of completeness, other records in the British Isles are included here. In Scotland, Wheat Bulb Fly is a well-known pest, but attacks do not occur on the same scale as in England. They are also mainly confined to the

drier eastern side where most of the wheat is grown, and attacks are most frequently reported in Mid- and East Lothian. There are also records for Berwick, West Lothian, Fife, Perthshire and Angus (E. Dunn, *in litt.*) and Stirling (S. D. MacLagan, *in litt.*). According to Morison (1955) the fly reaches its north-western limit of economic importance in south Kincardineshire though it does occur farther north.

There appear to be no records of the fly in Northern Ireland, but Dr. J. Carroll (*in litt.*) has informed me that wheat is occasionally infested in the Republic of Ireland, though not to such an extent that the yield is likely to be reduced.

Summary.

It is estimated that in 1953, as a result of attack by Wheat Bulb Fly, *Leptohylemyia coarctata* (Fall.), 59,000 acres of wheat were re-drilled and 58,000 acres were patched with spring-sown wheat; another 85,000 acres were affected without any action being taken by the farmer. The cost of new seed and the reduction of yield of spring wheat as compared with winter wheat represent a minimum financial loss of about £1,200,000. With the aid of maps it is shown that the distribution of damage is similar to the distribution of wheat and to the area with less than 30 in. rainfall annually. In districts where over 5 per cent. of the combined crops and grass acreage is in potatoes, there is a tendency for the amount of damage by Wheat Bulb Fly to increase with increasing potato acreage.

It is tentatively suggested on circumstantial evidence that high rainfall on the west side of the country is partly responsible for the absence there of damage by Wheat Bulb Fly. Many of the variations in the distribution of damage on the eastern side of the country could be accounted for by differences in cropping.

In Scotland, the distribution of the fly is also confined to the eastern side. There are a few records of the fly in the Republic of Ireland.

Acknowledgements.

Thanks are due to Advisory Entomologists in all parts of the country, particularly to Messrs. M. Cohen, W. E. H. Hodson, H. C. F. Newton, H. W. Thompson and J. A. White. The District and County Officers who supplied information to these and other Advisory Entomologists are too numerous to mention personally but without their help this work could not have been done.

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R.

A NEW SPECIES OF *HELOPELTIS* (HEMIPTERA—HETEROPTERA,
MIRIDAE) FOUND IN CEYLON.

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The earliest reference to the occurrence of *Helopeltis* in Ceylon was made by the French entomologist, Signoret (1858), when he described *Helopeltis antonii*, the so-called "Mosquito Blight". *Helopeltis* attacking cacao in Ceylon was first recorded in the year 1880 or 1881 in the Matale district (Green, 1901), the damage to the young leaves and stems having attracted the attention of cacao planters. Populations of *Helopeltis* occurring on cacao today, however, favour the pods, damage to young leaves and stems being relatively rare and generally associated with poor shade over the cacao plant. This apparent change of feeding habits prompted a careful and critical examination of the existing species of *Helopeltis* in Ceylon. Extensive collections of *Helopeltis* from the major cacao-growing areas in the island, including the Matale district, have not provided any specimens referable to *Helopeltis antonii*. The new species described below was the only species of *Helopeltis* found on cacao at Teldeniya, Kundasale and in the Matale district, which may be regarded as representative of the major cacao-growing areas of Ceylon.

***Helopeltis ceylonensis*, sp. n. (fig. 1).**

Colour.—Glabrous. Antenna: basal end of segment 1 whitish, rest deep brown to black. Head: shining black with whitish patches on genae and occipital region behind eyes. Pronotum: blood red to orange red; anterior lobe of pronotum posteriorly, posterior lobe anteriorly, suffused with piceous. Scutellum brownish to black; scutellar spine piceous narrowly whitish basally. Legs: base of femora black followed by whitish ring, rest deep brown to black. Hemelytra feebly infumate, iridescent; costal area of corium, cuneus, darker infumate. Abdomen: black with segments 3-5 or 6 whitish, remainder black. The thorax in some specimens is almost entirely piceous.

Structure.—Antenna: basal segment moderately thick with apex much thicker; very sparsely setose; a little longer than head, pronotum and scutellum together; segment 2 slender, approximately one quarter longer than segment 3; segment 4 half as long as segment 3, densely setose. Head: about twice as wide as long, measured across the eyes; postocular with a median elliptical foveole. Rostrum: extending to base of metasternum; basal segment extending almost to base of head. Scutellum: rounded posteriorly, depressed anteriorly to point of origin of scutellar spine. Scutellar spine feebly curved backwards; feebly constricted apically.

Measurements:—

	Male	Female
Total length	5.5 mm.	6.5 mm.
Length of hemelytra	3.75 mm.	5.0 mm.
Greatest pronotal width	1.25 mm.	1.5 mm.

In general structure and colour the female resembles the male but is larger.

Described from a series of males and females. Teldeniya, Ceylon, 10.v.1956 (M. D. De Silva).

Holotype ♂, and ♂ ♀ paratypes of *Helopeltis ceylonensis* have been deposited (2414)

in the Division of Entomology, Department of Agriculture, Peradeniya, Ceylon. Further paratypes are in the British Museum (Natural History), London.

H. ceylonensis differs from *H. oryx* Distant 1904, and *H. antonii* in respect of its more robust habitus and darker coloration. It differs from *H. oryx*, of which the type, a female, is deposited in the British Museum, in coloration; in *H. oryx* the base of the antennal segment and femora is narrowly reddish yellow, the head has a reddish stripe laterally below the eyes, the thorax is light

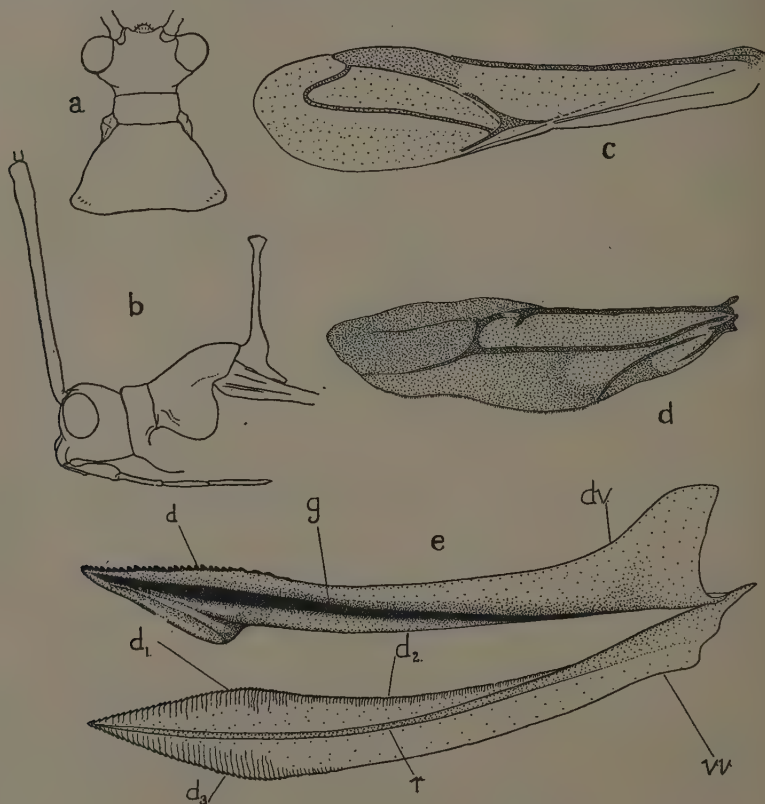


Fig. 1.—*Helopeltis ceylonensis*, sp.n. a, head and pronotum—dorsal view; b, head, pronotum, scutellum and scutellar spine—lateral view; c, hemelytron; d, metathoracic wing; e, constituents of ovipositor—dv, right outer aspect of right dorsal valve, vv, inner aspect of left ventral valve, g, groove, r, ridge, d, dentation.

red except the pleura which are reddish brown; segments 2–5 of the abdomen are light red, the remaining segments being shining black. The hemelytra are hyaline, not iridescent, with costal area and cuneus testaceous. In structure *H. oryx* differs in having a slender basal antennal segment, the apex of which is not very much thicker than the rest of the segment, more slender legs, and no foveole on the postocular.

H. ceylonensis differs from *H. antonii* in coloration, the relatively longer and thicker basal antennal segment, the more robust and less erect scutellar spine, more prominent antennophores and in having a foveole on postocular.

Acknowledgements.

The author is indebted to Dr. H. E. Fernando, Entomologist, Department of Agriculture, Ceylon, for suggesting a close examination of the species of *Helopeltis* damaging cacao in Ceylon today. He acknowledges with thanks the assistance of Mr. N. C. E. Miller of the Commonwealth Institute of Entomology, who not only helped him with the identification of *Helopeltis* specimens, but also read the manuscript and offered very valuable criticisms.

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A CONTROLLER FOR A LIGHT SOURCE.

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(PLATE X.)

Experimental work on the effects of increasing and decreasing light intensities on the activity and development of insects made it necessary to design a controller, working on a 24-hour cycle, that was capable of brightening and dimming a light source as well as controlling its "on" and "off" periods. The following flexibility was essential.

1. The period from full darkness to full light and *vice versa* to be adjustable from $\frac{1}{2}$ hour to 2 hours.
2. The period of full illumination to be adjustable from 8 hours to 16 hours.

The instrument was required to control a 200-watt, 240-volt, filament lamp and be capable of reliably repeating a large number of cycles of events.

The authors were advised that the desired control of a current of the order of 1 amp. by electronic means would be difficult, and it was also felt that the mechanical linkages of a motor-driven variable transformer, controlled by a time switch, would be too involved to be reliable, if the controller were to meet requirement 1. Moreover, such an arrangement would impose difficulties if subsequent modification of the brightening and dimming characteristics were desired.

The final design involves a minimum of moving parts, and the adjustments of 1 and 2, above, are very simple. The brightening and dimming operations are carried out in a number of small steps instead of in a smooth manner.

The controller (Pl. X, fig. 1) is built around a synchronous time-switch motor rotating once in 24 hours. To its spindle is attached an arm carrying a contact brush which is drawn across the faces of two "commutator blocks" connected to a tapped resistance unit. By varying the angle between the two commutator blocks, the total period of illumination is adjusted, and by altering the position of the contact brush along the length of the revolving arm the brightening and dimming periods are adjusted. One commutator block is fixed in the zero-hour position and serves as the brightening control. The other block serves as the dimming control and is mounted on an arm movable around the same centre as the motor spindle. Calibration of the circle as shown enables very simple adjustment of the "full on" period to be made.

The commutator blocks through which the lamp circuit is completed and which lead to the tapped resistance unit are of such dimensions that the arc swept by the moving brush subtends an angle of $7\frac{1}{2}^\circ$ at the position farthest from the centre and an angle of 30° closest to the centre. These angles represent $\frac{1}{2}$ hour and 2 hours, respectively, and any period between these limits is selected by moving the brush holder to the appropriate position along the length of the rotating arm. Both blocks are so mounted that the "full on" segments lie radially.

Construction details.

The commutator blocks consist of a number of parallel copper wires insulated from one another and cemented in position. They were made by milling out a

channel, $1\frac{3}{8}$ -in. wide and $\frac{1}{8}$ -in. deep, in the face of a piece of heat resisting insulating material, $4\frac{1}{2}$ -in. long and $\frac{1}{4}$ -in. thick. The two ends of the channel were rounded and in it was wound a single-layer coil of 30 turns of 20 S.W.G., D.C.C. copper wire, which completely occupied the width of the channel. The assemblies were then heated and Araldite Type 1 adhesive resin applied to the wires in the channel only. Sufficient resin was applied to soak through the cotton insulation and fill the space between the underside of the wires and the block. After clamping the wires flat the assembly was cured at 200°C .

The surface was then milled away to a depth of half the diameter of the wire, and the leading edge of each block was chamfered to assist the reception of the moving contact. Each turn of the coil was cut at the back of the block and one end of each wire lifted away to serve as a connection to the tapped resistance unit.

If carefully made in this manner, the commutator blocks will provide a good area of electrical contact, and will possess a very high resistance between each segment. It is important that the copper wire used for the winding must not be enamelled: if it is, only the enamel will be cemented to the base and the adhesive resin will not hold the copper.

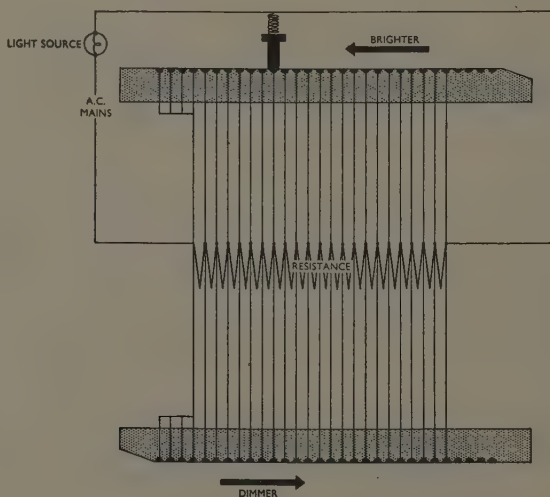


Fig. 1.—Circuit diagram.

Both blocks are connected to a single resistance unit as shown in fig. 1. In the present instance the resistance is provided with 22 tapplings of 43 ohms each. This gives a potential of approximately 11 volts between neighbouring segments of the commutator blocks, as high as is advisable if sparking is not to occur. Steps of this size provide a sufficiently smooth brightening and dimming operation.

To maintain the "full on" current through the lamp, between the brightening and dimming controls, a further circuit is required. The arrangements for this are shown in Plate X, fig. 2. A fixed contact is mounted close to the spindle end of the revolving arm and, under the contact, a split copper ring is attached to the "full on" segments of the dimming control block. The ring is free to slide in a shallow, right-hand, helical groove built into an insulating ring of perspex. At the position corresponding to "full on" of the brightening control

the perspex shroud ends and exposes the copper ring. Thus the "full on" lamp current is always maintained for the correct period and requires no separate adjustment.

The commutator blocks possess 30 segments, whereas the resistance unit has 22 tappings only. The extra segments are placed equally on either side of the effective ones. As the main contact brush moves on to the brightening control it is able to bed down on to the four idle segments before carrying any current. This is important if sparking is to be avoided. The idle segments at the trailing edge of this control and at the leading edge of the dimming control are all connected to the "full on" tapping of the resistance unit. This arrangement provides a reasonable distance of travel of the arm during any part of which the fixed inner brush can make or break contact with the helical copper strip. In this way any need for high accuracy, during construction, in the positioning of the contacting components is eliminated. Current is fed to the arm assembly by means of the brass ring and horizontal contact shown in Plate X, fig. 2.

It was found that sparking between the contact and the commutator blocks could be entirely suppressed providing care was given to the following factors:

1. The main brush to be spring-loaded and made of solid copper.
2. The diameter of the brush to be sufficient to straddle two neighbouring commutator segments.
3. The surface of the contact and of the two commutator blocks to be given a fine, honed finish and kept clean by the use of a dust-proof cover over the whole assembly.
4. Open circuit condition to be avoided by permanently connecting the brush to the last tapping on the resistance unit as shown in fig. 1. Under these conditions a small current passes through the lamp during the "full off" period but this is not sufficient to cause the filament to glow. The arrangement avoids the build-up of a 240-volt potential between the brush and the first effective commutator segment.

The contact pressure did not appear to be critical and it was only necessary to avoid falling below a certain minimum determined by trial.

The controller has proved reliable in service in studies of the effects of changing light intensity and photoperiod on the activity and development of insects. Any modifications of the brightening and dimming characteristics that may be desirable later will require only the substitution of an alternative resistance unit.

Summary.

An instrument is described that will brighten and dim a light source and control its "on" and "off" timing.

The periods of brightening and dimming and the period of full illumination are both adjustable.

The instrument is being used successfully for studying the effects of changing light intensity and photoperiod on the activity and development of insects.

Acknowledgements.

The authors wish to thank Mr. F. W. Buckley who constructed the instrument and contributed many useful suggestions, and Mr. L. A. Marshall who prepared the figures.

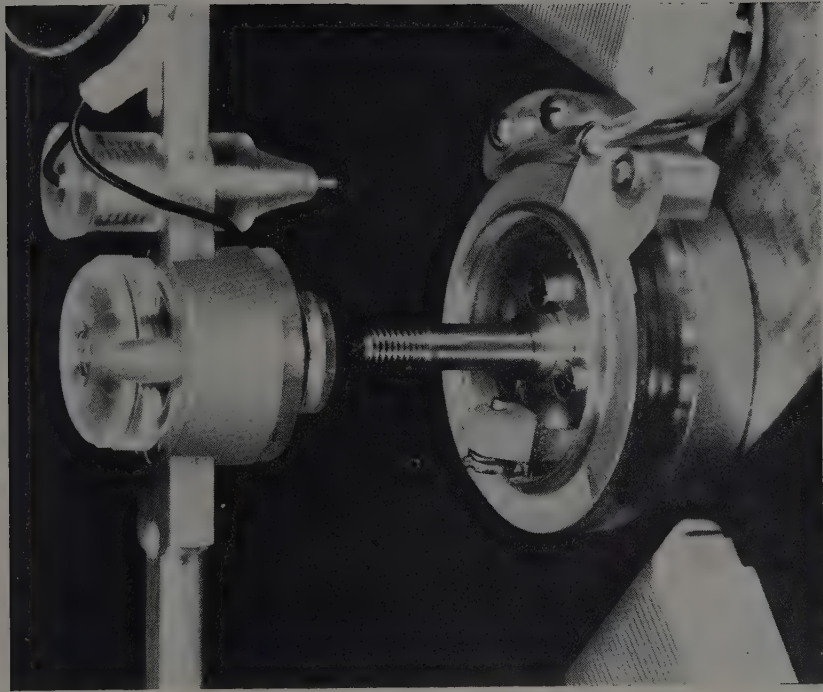


FIG. 2. Details of arrangement for maintaining "full on" lamp current and for supplying current to revolving arm.

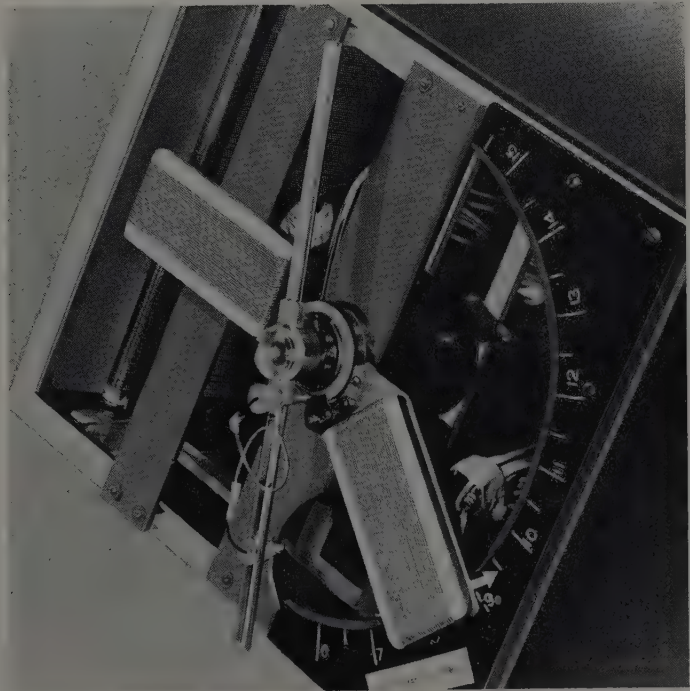


FIG. 1. General arrangement.

OBSERVATIONS ON THE REPRODUCTIVE BEHAVIOUR OF
AMPHOROPHORA RUBI (KALT.), WITH SPECIAL
 REFERENCE TO THE PHENOMENON OF
 INSECT RESISTANCE IN RASPBERRIES.

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In 1947, *Amphorophora rubi* (Kalt.) was shown conclusively to be a vector of European virus diseases of *Rubus* in Britain (Cadman & Hill, 1947). In North America, this species had been known for a number of years to transmit viruses of *Rubus*, and early investigations were made there into the possibility of restricting the spread of the virus diseases by employing varieties of raspberries upon which the vector feeds with difficulty or not at all (Schwartz & Huber, 1937; Huber & Schwartz, 1938; Schwartz & Huber, 1939; Schwartz, 1945; Anon., 1946). This work apparently met with considerable success, for the optimistic statement was made "Breeding varieties that transmit this immunity are being developed to such an extent that in the future new varieties may be entirely free from the dangerous disease" (i.e., *Rubus* mosaic) (Anon., 1946).

Schwartz & Huber (1937) tested some ten varieties of red raspberries and found that Antwerp, Herbert and Newburgh showed marked resistance, while Lloyd George was completely resistant. In subsequent experiments (Huber & Schwartz, 1938) these results were confirmed and additional varieties were also graded. Numerous hybrids of Lloyd George crossed with susceptible varieties were examined at the same time. The results of these experiments indicated that a variety may be completely resistant to *A. rubi* without being homozygous for resistance. Examination of inbred seedlings of Lloyd George revealed that variety to be heterozygous for resistance. In a further paper (Schwartz & Huber, 1939) it was stated that there were strong indications that Lloyd George carries two or more factors for resistance and that resistance is dominant to susceptibility.

Dutch workers (Kronenberg & de Fluiter, 1951) made observations on a series of raspberry varieties to determine which of those grown commercially in Holland showed signs of resistance to *A. rubi*. In their report, they segregated the varieties into three groups: (1) resistant, (2) partially resistant, (3) susceptible.

The present paper gives an account of observations made in Scotland on the reproductive behaviour of *A. rubi* on varieties of *Rubus idaeus* and other species of *Rubus*. The preliminary experiments, described first, were performed in order to obtain a general idea of differences in varietal resistance. The second set of experiments was made to analyse more precisely how the reproductive behaviour and the development of the Aphids were altered on varieties showing differing degrees of resistance.

Materials.

Plants derived from the following species of *Rubus* were tested: *R. idaeus*, *R. strigosus*, *R. occidentalis*, *R. Lambertianus*, together with hybrids of *R. idaeus* × *R. strigosus* and *R. idaeus* × *R. fruticosus*.

Rubus idaeus group: Malling Enterprise, Malling Jewel, Malling Promise, Malling Landmark, Norfolk Giant, St. Walfried, Lloyd George, seedlings 64/2, 69/189, 72/59.

Rubus occidentalis group: Cumberland.

Rubus strigosus group: *R. strigosus*, Newburgh, Latham.

R. idaeus × *R. strigosus*: Viking.

R. idaeus × *R. fruticosus*: loganberry.

The plants were raised from root-cuttings in shallow boxes containing sterilised John Innes seedling compost. When they had grown to about two inches in height they were transferred to pots containing John Innes compost.

The stocks of *A. rubi* were raised from a single female collected from a wild bramble. All the experiments were performed in a gauze-walled, unheated insectary.

Preliminary Experiments.

In these experiments, eight varieties of raspberries currently grown in commercial plantations in Great Britain were tested, namely, Malling Promise, Malling Jewel, Malling Enterprise, Malling Landmark, Norfolk Giant, St. Walfried, Viking and Newburgh; also included were the American varieties Cumberland and Latham, nameless seedlings, raised by Mr. Norman Grubb at East Malling, numbered 64/2, 69/139 and 75/59, respectively, loganberry, *R. strigosus* and *R. Lambertianus*.

Five plants of each variety or species were first fumigated with nicotine vapour to ensure that they were initially free from insect life. Each plant was then covered entirely with a fine muslin sleeve supported by canes. After an adequate airing period had elapsed, one freshly mature female of *A. rubi* was placed on the young leaves near the growing point of each plant; care was taken to ensure that all Aphids were of the same age. The cages were then closed and allowed to remain undisturbed for 28 days. They were arranged in a random manner in the insectary in order to minimise positional effects.

At the end of the test period the cages were opened and all the Aphids therein were collected and preserved in alcohol for subsequent counting in the laboratory. These preliminary observations were made during the summer.

Results.

In Table I are given the average numbers of progeny produced during the period of the experiment on each variety and species of *Rubus*. It is clear that

TABLE I.
Number of progeny produced by one freshly mature female Aphid in 28 days
on different varieties of *Rubus*.

Variety	Av. no. progeny	Variety	Av. no. progeny
Malling Enterprise ..	359.8	69/139	34.0
Malling Jewel	211.6	Latham	9.6
Malling Promise ..	193.6	Newburgh	0.2
Cumberland	119.0	Malling Landmark ..	0
Norfolk Giant ..	112.4	64/2	0
St. Walfried	105.2	Loganberry	0
72/59	50.8	<i>R. strigosus</i>	0
Viking	34.2	<i>R. Lambertianus</i> ..	0

there is a considerable variation in the susceptibility of the different plants to the aphid attack.

Malling Enterprise, Malling Jewel and Malling Promise are most susceptible. Cumberland, Norfolk Giant and St. Walfried show a somewhat lesser degree of susceptibility yet carry quite heavy aphid populations. The seedlings 75/59 and 69/139, Viking, Latham and Newburgh show a distinct and increasing degree of resistance to the insects. Complete resistance is reached in the variety Malling Landmark and the closely related seedling 64/2. Loganberry, *R. strigosus* and *R. Lambertianus* also failed to support the Aphids. Thus the varieties and species tested can best be arranged in a graded series showing decreasing susceptibility rather than in the clearly defined groups which Kronenberg & de Fluiter established (1951).

Having established this range of resistance to *A. rubi*, further experiments were planned to analyse in greater detail the interrelationship of plants and Aphids in order to obtain more precise information as to how the reproductive activities, etc., of the Aphids are altered when they feed on certain varieties of raspberry.

Further Analysis of Resistance.

Comparative records were made to determine the effect of resistance on fecundity, reproductive rate, length of life, growth rate and behaviour of the Aphids.

As these experiments involved detailed observations, the number of raspberry varieties employed was reduced to four—Lloyd George, Malling Promise, Newburgh and Malling Landmark. Although Lloyd George had not been tested in the preliminary experiment it was decided to include it in the detailed analysis partly because it is one of the most popular British commercial varieties, but mainly because Huber & Schwartze (1938) had described it as completely resistant to *A. rubi*, a statement in no way supported by field and insectary observations made by numerous workers in Britain. Also, as the Dutch workers did not include Lloyd George in their test series, it seemed advisable to obtain detailed information about the interaction of this interesting and important variety with *A. rubi* in Europe.

The experiments were conducted in July and again from late September until mid-November so that any seasonal variations in resistance might be detected.

Instead of enclosing the whole plant in a muslin sleeve as before, bi-valve leaf-cages made of perspex tubing ($\frac{1}{2}$ " diameter) were used (Hill, 1955). One

TABLE II.

Total progeny produced by one freshly mature female Aphid on different varieties of *Rubus*.

Variety	Total progeny			
	Summer		Autumn	
	Mean of five	S.E.	Mean of five	S.E.
Lloyd George	31.6	1.913	18.8	3.611
Malling Promise ..	26.8	2.131	21.2	6.711
Newburgh	14.2	1.655	11.6	4.675
Malling Landmark ..	3.0	1.304	4.4	6.439

cage was clipped to the second expanding leaf behind the growing point of each plant, with the muslin-covered valve on the under-surface of the leaf. Within each cage a single female Aphid was placed. Care was taken again to ensure that all the Aphids were of the same age.

There were five replicates of each variety and the plants, which were in pots, were again arranged in a random manner in the insectary.

Observations on the fecundity of A. rubi.

Daily or two-daily counts were made of the numbers of nymphs produced by each adult Aphid; great care was exercised to disturb the insects as little as possible, and the nymphs were left *in situ*. If, however, an individual were to mature before the end of the experiment, it would be removed before it started to reproduce. Observations were continued until the adult female died, or, in the case of the summer colonies on Lloyd George, until the condition of the leaf indicated that further counts might not give accurate results.

In both the July and September experiments, Lloyd George and Malling Promise do not differ significantly in the populations of nymphs produced on them (see Table II). The figures suggest that in summer Lloyd George may be slightly the more suitable for the Aphid. The reverse, however, is suggested by the autumn figures. Although statistical significance is not achieved by these

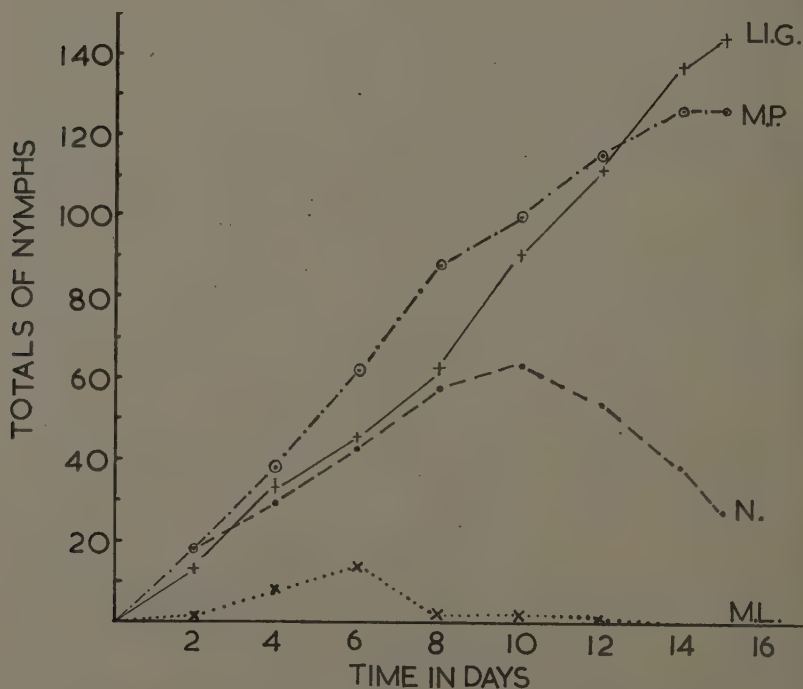


Fig. 1.—Increases in total populations of nymphs on four raspberry varieties in the summer experiments. L.G., Lloyd George; M.P., Malling Promise; N., Newburgh; M.L., Malling Landmark.

differences it should be noted that parallel results were obtained in the observations on the comparative lengths of life of the adult Aphids on these two varieties (see below). This would indicate that some slight difference may exist between these varieties and that they may also show a differential seasonal change, perhaps of a biochemical nature. To have clarified this point it would have been necessary to perform more extensive experiments than were possible in the limited space available.

Newburgh gave results of considerable interest. In the preliminary experiments this variety appeared to be resistant, but under the conditions of the present experiment the Aphids did succeed in reproducing on it both in summer and autumn. This corroborates to some extent the results reported by Kronenberg & de Fluiter who stated that "... these varieties [of which Newburgh was one] though appearing rather resistant in the field under certain circumstances, are not resistant under *all* conditions."

The difference between the populations on Malling Landmark and those on the other varieties is highly significant at both seasons. Malling Landmark can be regarded as a variety showing a very marked degree of resistance. The Dutch workers, however, reported that on varieties such as Malling Landmark they had noted that the Aphids did show an ability to thrive rather well on the old yellow leaves of the plants. In the present experiments also, there is a distinct indication that some plants of Malling Landmark do allow the Aphid to multiply on them during the autumn, the season of senescence when the general physiology of the plants may approach a condition similar to that existing in a yellowing leaf. This statement is also supported by a number of field observations where small populations of *A. rubi* have been observed on Malling Landmark towards the end of the summer.

Differences in the reproductive rate of the Aphid.

Graphs of the total populations of nymphs on the four raspberry varieties are shown in figs. 1 and 2. It is clear that in both summer and autumn the varieties fall broadly into two groups; one contains Lloyd George, Malling Promise and Newburgh; the other contains Malling Landmark. Within the first group, however, Newburgh is seen to differ somewhat from the other two varieties in that, although populations of nymphs on it increase at first almost as rapidly as on Lloyd George and Malling Promise, yet in summer an early cessation of increase is evident, due to the premature death of the mothers. In addition, the population level of the nymphs is not maintained because of their fairly rapid death.

The graph of the autumn increase in population of nymphs (fig. 2) also suggests that Newburgh may be slightly less suitable for aphid reproduction than the other two varieties in the group. However, the difference at that season does not appear to be so great as in summer, possibly again indicating a seasonal change in the plant's physiology which is of advantage to the insect. Malling Landmark is once more significantly more aphid-resistant than the other varieties. It is clear from comparison of the two graphs, however, that in autumn this variety also becomes altered in some way which is to the insect's advantage, as a small number of nymphs was produced over an extended period of time, and one of these nymphs even succeeded in reaching maturity (see below).

Length of life of adult Aphids.

Records were kept of the length of life of each of the original mother Aphids on the four varieties. In the summer experiments, all the mothers on Malling Landmark and Newburgh died at a much earlier age than did those on Lloyd George and Malling Promise, indicating again the existence in the former

varieties of factors unfavourable to Aphids. An interesting difference between the latter two varieties appeared in the death of all the mothers on Malling Promise before the end of the experiment while all the mothers on Lloyd George were still alive. It should be noted, however, that, while the latter out-lived

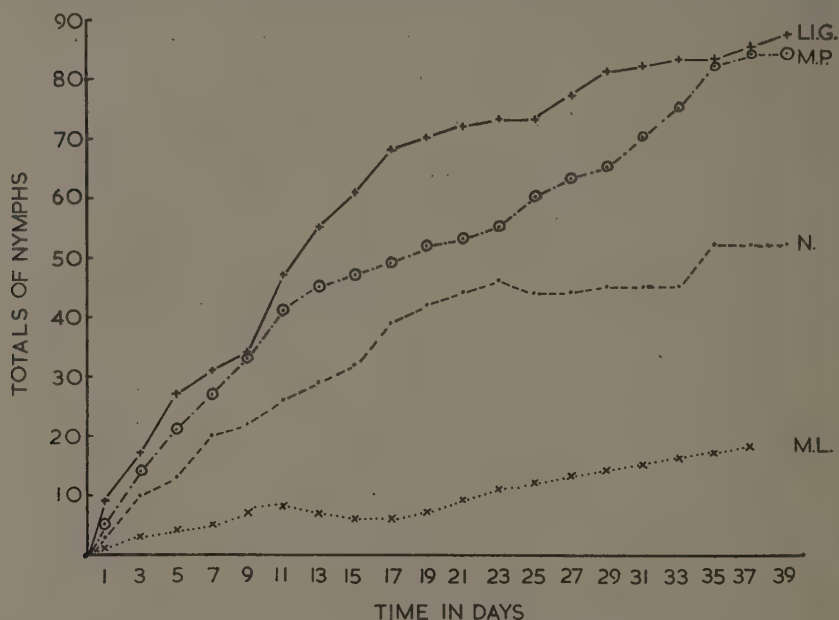


Fig. 2.—Increases in total populations of nymphs on four raspberry varieties in the autumn experiments. Varieties as in fig. 1.

the former, they also reproduced more slowly and were obviously near to the end of their reproductive lives when the experiment was terminated.

In the autumn, the results from Lloyd George and Malling Promise were reversed; females on the latter outlived those on Lloyd George. Again, the

TABLE III.

Length of life of adult Aphids on different varieties of *Rubus*.

Variety	Length of life (days)			
	Summer		Autumn	
	Mean of five	S.E.	Mean of five	S.E.
Lloyd George	21.6	1.40	28.4	3.501
Malling Promise ..	14.8	0.735	34.6	7.947
Newburgh	10.8	0.490	18.6	6.361
Malling Landmark ..	7.8	0.490	13.4	6.608

females on Newburgh and Malling Landmark showed a shorter length of life than did those on the other two varieties, but the mean values were somewhat higher than those recorded in the summer. The high mean figure of 13.4 days given for Malling Landmark was achieved, however, because one female greatly outlived the other four on that variety, the mean of whose lengths of life was 7.0 days. This possibly indicates the presence of important variations in the genetical constitution of either the insect or the plant, although clonal stocks of both were used. Such variations are not uncommon among Aphids (Bonnet-maison, 1951) and constitute an important factor in all work connected with aphid resistance in plants.

Growth rate of nymphs.

It was found possible to keep track of individual nymphs because, where reproduction was most rapid (*i.e.*, where the plant was most suitable), the nymphs remained stationary and their positions in the cages could be noted.

The number of days required by the first-born nymph in each cage to complete its development to the final ecdysis was used as a standard for assessing the

TABLE IV.

Time, in days, taken by first-born nymph to reach adult form on different varieties of *Rubus* in summer.

Variety	Replicates					Mean	S.E.
	1	2	3	4	5		
Lloyd George	19	16	21	21	19	19.2	.917
Malling Promise ..	16	16	19	19	19	17.8	.734
Newburgh	—	—	—	—	—	—	—
Malling Landmark ..	—	—	—	—	—	—	—

effect of the different varieties on the growth rate of the aphid nymphs. In summer all the first-born nymphs on Lloyd George and Malling Promise survived, and the periods required to reach the adult stage on these two varieties do not differ significantly (Table IV).

There is, however, an indication that on Malling Promise the growth rate may be slightly more rapid. All the nymphs produced during the summer on

TABLE V.

Time, in days, taken by first-born nymph to reach adult form on different varieties of *Rubus* in autumn.

Variety	Replicates					Mean	S.E.
	1	2	3	4	5		
Lloyd George	26	27	29	30	27	27.8	0.734
Malling Promise ..	28	28	25	28	35	28.8	1.655
Newburgh	27	27	32	—	26	22.4	5.697
Malling Landmark ..	—	—	—	—	28	5.6	5.600

Newburgh and Malling Landmark died before reaching maturity. Those on Newburgh lived longer than those on the other variety, but, without exception, they died in the early instars.

In autumn, an interesting change was observed in the much higher survival rate of nymphs on Newburgh. Not only did they mostly survive long enough to reach the adult stage but their growth rate was clearly comparable to that of nymphs on Lloyd George and Malling Promise (Table V). Indeed one of the first nymphs to become adult in the autumn experiments was feeding on a Newburgh plant. In the case of Malling Landmark, mortality of nymphs was again high but one nymph succeeded in becoming adult and, again, it did so as rapidly as some on the other varieties.

The behaviour of adult female Aphids.

Marked differences in the behaviour of the adult female Aphids were observable immediately after they had been placed in the bivalve cages at the commencement of the experiments. On Lloyd George and Malling Promise all the females settled down quickly and proceeded to feed, and reproduction took place within a day. Most of them remained with their stylets inserted at one place; their progeny likewise formed up in lines near to them and fed quietly.

On Malling Landmark the adult Aphids seldom settled down but showed marked restlessness. Some of them were observed with their stylets in the feeding position but even these died in a few days. Exceptions to this occurred in the autumn experiments where one adult female survived for an exceptionally long period and some of her progeny also were able to feed, one of them ultimately becoming adult.

The females placed on Newburgh plants did attempt to feed after an apparent initial inability to find suitable food. As has been noted already (p. 471), these Aphids succeeded in producing nymphs, but very frequently, when the cages containing them were opened, both adults and nymphs were found to be off the leaf surface and clinging to the perspex or muslin. It was obvious that they did not find in Newburgh a satisfactory food-plant, especially during the summer. It is interesting to note that in the preliminary experiments, where the mother Aphids were not confined to the leaf surface by a small cage, Newburgh appeared to be resistant. It now seems most probable that under these conditions the mother Aphids, finding Newburgh unsuitable, wandered in search of satisfactory food and in so doing, dropped off the plants and died, thus leaving very few nymphs, most of which also died quickly. When they were prevented from wandering off the leaf surface by the bivalve cage, they did succeed in feeding and reproducing to a limited extent.

In the field, it is most likely that any individuals of *A. rubi* alighting on Newburgh plants would behave as did those in the preliminary experiments, so that the status of Newburgh in the field may well be more accurately indicated by the results of the preliminary experiments than by those of the more detailed analysis. This agrees with observations made by Kronenberg & de Fluiter (1951). In autumn, the reluctance of *A. rubi* to settle on Newburgh leaves, although less marked, was still to be observed.

Discussion.

That certain varieties of *Rubus idaeus* show resistance to the virus vector, *A. rubi*, has been demonstrated conclusively on a number of occasions. Resistance seems to involve a slowing down of the reproductive rate, a shortening of the length of life of the mother Aphids and of their progeny and prevention of the development of the nymphs. The fact that the degree of resistance of the plant is lowered in autumn, and also when the Aphids are feeding on senescent leaves, would indicate that resistance is bound up with the biochemical

constitution of the plant sap. No information exists as to how this biochemical resistance operates. The observation of Huber & Schwartz that *A. rubi* will not breed on the variety Lloyd George in North America is of considerable interest. The writer recently had the opportunity of testing North American material of *A. rubi* on plants of commercial varieties, including Lloyd George, grown from root cuttings imported from Britain to Canada for the experiment. He was able to confirm the statement of Huber & Schwartz, and, in addition, showed that the North American form of the Aphid, in contrast to the European, is capable of breeding very successfully on Malling Landmark. This clearly indicates the existence of at least two races of *A. rubi*, which differ markedly in their ability to utilise certain raspberry varieties for food (Hill, 1956).

In spite of the early optimistic reports, it is still problematical as to how practical would be the use of aphid-resistant varieties as a measure for limiting the spread of virus diseases of *Rubus*. The fact that the Aphids can feed and even survive and multiply on senescent tissues of resistant varieties casts further doubt on the usefulness of the latter. With what ease viruliferous individuals can successfully transmit the pathogens to resistant varieties during the short exploratory feeding probes which they have been observed to make is unknown. Information is also required about the fate of viruses injected into senescent leaves in the summer and into plants approaching their dormant phase in the autumn. A further aspect requires careful consideration, namely, how practical is it to present an aphid vector, which constantly breeds in reservoirs of wild plants, with fields of cultivated plants which it will find unsuitable as a source of food? The effect of this might well be to cause the Aphids to continue moving through the fields, testing further plants. Should the aphid population contain a percentage of viruliferous individuals, the result might be a very successful dissemination of the virus throughout the plantation, resulting in the achievement of the reverse of what was intended.

Summary.

Sixteen varieties of *Rubus*, comprising four species, were tested with the Aphid, *Amphorophora rubi* (Kalt.), which is a vector of virus diseases of *Rubus* in Europe and N. America, to establish the range of resistance to the development of populations of the insect. The phenomenon of resistance was then further investigated from the aspect of fecundity, reproductive rate, length of life, growth rate and behaviour of the Aphid. In these latter investigations only four varieties of raspberry were employed. The varieties Malling Promise and Lloyd George showed no resistance. Malling Landmark proved to be highly resistant but showed a slight reduction in resistance in the autumn. Newburgh appeared to occupy an intermediate position between Malling Landmark and the other two varieties. There were indications, however, that under field conditions its degree of aphid resistance may be greater than was observed under experimental conditions. Resistance seems to involve a slowing down of the reproductive rate, a shortening of the length of life of adults and prevention of development of the nymphs. The practicability of using aphid-resistant varieties of raspberry to minimise virus spread is discussed.

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EPHIALTES BREVICORNIS (GRAV.) AS AN EXTERNAL PARASITE
OF THE DIAMOND-BACK MOTH, *PLUTELLA*
MACULIPENNIS (CURT.).

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This paper gives an account of the life-history of *Ephialtes brevicornis* (Grav.) (ICHNEUMONIDAE) as a parasite of *Plutella maculipennis* (Curt.) with a description of the various instars and their head capsules.

Thorpe (1930) has previously illustrated the head capsule of the final-instar larva of *E. brevicornis* and has also given some information on it as a parasite of *Rhyacionia buoliana* (Schiff.). It is thought, however, that figures and descriptions of all the instars will be useful, as Thorpe's illustration of the head capsule of the final instar is somewhat diagrammatic.

Host Records and Distribution.

Morley (1908) has described the adults of *E. brevicornis*. This species has commonly been recorded in the literature as *Pimpla brevicornis*; for a reference to synonymy see Perkins (1943). Morley states that the females of *E. brevicornis* are very common everywhere throughout central and northern Europe but that the males are always uncommon. Thorpe (1930), however, found that in material reared by him he obtained 82 males and 6 females. Although in the present study the sex ratio was not accurately determined due to lack of numbers, the males were in the majority.

The list of hosts is very large and covers a wide range of families. *E. brevicornis* has previously been recorded from eight families of the Lepidoptera (Morley & Rait-Smith, 1933) and from one family of the Hymenoptera (Thorpe, 1930). Hosts from which it has been recorded as having been reared are as follows:—

LEPIDOPTERA

Pieridae	<i>Leptidea sinapis</i> (L.)
Noctuidae	<i>Harmodia rivularis</i> (F.)
Geometridae	<i>Eulype</i> (<i>Melanippe</i>) <i>hastata</i> (L.)
	<i>Eupithecia linariata</i> (Schiff.)
Tortricidae	<i>Cacoecia rosana</i> (L.)
	<i>Tortrix forsterana</i> (F.)
	<i>Laspeyresia</i> (<i>Coccyx</i>) <i>cosmophorana</i> (Treitschke)
	<i>Polychrosis</i> (<i>Sericoris</i>) <i>euphorbiana</i> (Frey.)
	<i>Evetria resinella</i> (L.)
	<i>Phalonia flaviciliana</i> (Westw.)
	<i>Rhyacionia buoliana</i> (Schiff.)
Tineidae	<i>Luffia lapidella</i> (Goeze)
Gelechiidae	<i>Aristotelia</i> (<i>Doryphora</i>) <i>pulveratella</i> (H.-S.)
Coleophoridae	<i>Coleophora frischella</i> (L.)
Elachistidae	<i>Tischeria complanella</i> (Hb.)

HYMENOPTERA

Tenthredinidae	<i>Ardis brunniventris</i> (Htg.)
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Ephialtes has not previously been recorded from *Plutella maculipennis* though it probably occasionally parasitises this host in nature.

Methods and Materials.

Five thousand parasitised larvae of *Coleophora frischella* were collected from *Melilotus altissima* at Montmédy, France, by the European Laboratory of the Commonwealth Institute of Biological Control, and consigned to New Zealand by air in August 1955. The parasites expected to emerge from this material would have been used to control what was believed to be *C. frischella*,* and perhaps *C. spissicornis* (Haw.) in New Zealand. Three females and five males of *E. brevicornis* emerged from the Montmédy material during September 1955 and these and their progeny constituted the material used in this work.

The three original females were offered final-instar larvae of the *Coleophora* spp. occurring in New Zealand but these were ignored. It was then found that they would parasitise the prepupae of *P. maculipennis*.

A constant supply of host material of *P. maculipennis* was easily obtained by rearing this insect on cabbages in a heated glasshouse. It might be mentioned here that *P. maculipennis* is an ideal insect for studies on external parasitism. The cocoon formed by this insect is composed of a fine, loosely woven mesh through which the prepupa and pupa can be easily seen. It thus allows inspection of external parasites with the minimum of disturbance.

Individual females of *E. brevicornis* were placed with several males in horizontal 4" x 1" glass tubes sealed at one end by fine muslin and at the other by a cork. The insects were fed on a mixture of honey and water.

Cocoons of *P. maculipennis* on small pieces of cabbage leaf were placed in these tubes and females of *Ephialtes* were allowed to oviposit in them. The cocoons were inspected frequently under a stereoscopic microscope and any seen to be parasitised were immediately transferred to a small petri dish and placed in an incubator. The incubator was run at a temperature of 20°C. and the humidity was controlled at 60 per cent. R.H. by storing the petri dishes over a KOH solution in a desiccator. The dishes were removed at regular intervals and the developing eggs and larvae of *Ephialtes* inspected. The number of instars was determined by inspection and checked by counts of head capsules.

Larvae used in the description of the various instars were preserved in 90 per cent. alcohol after having first been fixed in K.A.A.D. mixture (Peterson, 1951). Preparations of head capsules were made by first staining in Ehrlich's haematoxylin and then mounting in Berlese's fluid. This technique proved quite satisfactory and quick, though the delicate purple of the haematoxylin stain was not obtained.

Drawings of the whole larvae and head capsules were made with the aid of a camera lucida.

General Bionomics.

In Europe, the adults of *E. brevicornis* are found from the end of June till the end of September, being most common towards the end of August (Morley, 1908). As its list of hosts is very extensive it is likely that the long flight period is connected with this. No field observations were made in New Zealand as *Ephialtes* was not liberated.

E. brevicornis in the laboratory is quite long-lived. Females fed on honey and water survived for about six weeks at ambient temperatures (approximately 60°F.). Males lived for a somewhat shorter period. During this time both males and females were extremely active.

* Of the two species of *Coleophora* in New Zealand only *C. spissicornis* (Haw.) is identified without doubt. While the systematic position of the other species is obscure, it is definitely not *C. frischella* (L.).

In addition to feeding on the solution of honey and water, adult females of *E. brevicornis* feed extensively on the body-fluids of the host. They do this after an inspection of the *Plutella* cocoon similar to that described below for oviposition. The prepupa or final-instar larva of *Plutella* is stung until all movement in it ceases. The female then proceeds to gnaw through the flimsy cocoon, its ovipositor still remaining in the *Plutella* larva. The puncture made by the ovipositor is enlarged by the mandibles, and the ovipositor being withdrawn, the body fluids of the host are consumed to a greater or lesser extent. Quite often, within a short period, many larvae or prepupae of *Plutella* may be stung and only partly consumed.

The abdomens of the males and females of *E. brevicornis* on emergence are distinctly compressed. After feeding on honey and water, which may begin immediately, the abdomen becomes distended. No egg-laying was observed until approximately 7 to 10 days after emergence, although feeding on the host may occur before this. It is by no means certain that feeding on the body-fluids of the host is essential to maturation of the eggs but it will probably prove at least to speed up their maturation (Lloyd, 1940; Edwards, 1954). It would seem that in this species of *Ephialtes* this distinctly predacious habit has developed from oviposition behaviour as suggested by Clausen (1940, p. 72).

Mating occurs immediately after emergence though it is repeated throughout the adult life of both males and females. The active male first faces the female with his wings vibrating and emitting, sporadically, a high-pitched buzz of short duration. He then mounts upon the back of the female and, with his wings still vibrating, curves his abdomen round and under hers to effect copulation. The pair are in copula for only a few seconds though the process may occur again and again within a very short period. Males have been seen attempting to copulate with females actively engaged in oviposition.

When fresh cocoons of *P. maculipennis* are introduced into a rearing tube containing a female of *Ephialtes*, she is immediately attracted to them. It should perhaps be mentioned that adults of *Ephialtes* are positively phototropic when in the rearing tubes, but the stimulus of host material neutralises and replaces this reaction. The female approaches a cocoon and inspects it by walking around it and over it. Her antennae are curved downwards and tap the cocoon constantly. After the preliminary examination, the female straddles the cocoon lengthwise, withdraws her ovipositor from its sheath and with the tip of her abdomen high in the air inserts her ovipositor through the mesh of the cocoon into the *Plutella* larva or prepupa. She may find it necessary to thrust her ovipositor more than once into the cocoon until the cuticle of the larva is pierced. The *Plutella* larva or prepupa moves quite actively when this happens but all movement soon ceases. It should be noted here that a distinct preference is shown by the female for the prepupa of *Plutella* rather than the final-instar larva or pupa. Eggs were found extremely rarely on final-instar larvae and never on pupae of *Plutella*. This preference seems to be due to the fact that the prepupal stage of *Plutella* is relatively immobile yet the cuticle is not quite so hard as in the pupa. The ovipositor of *Ephialtes* seems unable to penetrate the hard cuticle of the pupa, and although it can penetrate the cuticle of a final-instar larva which has just completed a cocoon, the larva invariably commences to move violently when attacked and more often than not leaves its cocoon and escapes.

After the *Plutella* larva or prepupa has been paralysed, the female *Ephialtes* withdraws her ovipositor, usually turns around, and thrusts her ovipositor through the mesh of the cocoon and deposits an egg beside the larva or prepupa of *Plutella*. During this action the ovipositor projects forward at a very acute angle until it is almost parallel to the body. The time for the completion of the whole operation of ovipositing was from 2-4 minutes, and the time from when the ovipositor was placed in position for oviposition till the egg was deposited averaged 45 seconds.

Usually only one egg is laid in a cocoon but occasionally two or three may be found and on one occasion, when *Plutella* prepupae were scarce, 12 were found in one cocoon. Now and again eggs were found outwith the cocoons but this is abnormal.

The egg is somewhat sticky and adheres to the body of the paralysed host, the outside of the cocoon, or the surface of the cabbage leaf. When kept at 20°C. and 60 per cent. R.H., the eggs hatch in approximately 36 hours after being laid. Never more than one larva was ever seen to survive in a cocoon no matter how many eggs had been laid. Death of any excess appeared to be due to intraspecific competition.

The first-instar larva on hatching immediately begins to feed on the body-fluids of the paralysed host. Sometimes, however, if the egg has been laid outside the *Plutella* cocoon, the larva may wander away and die. It would appear, therefore, that there is no strongly developed host-finding reaction in the larva. When feeding, the larva never completely enters the host and it can always be seen easily through the almost transparent mesh of the *Plutella* cocoon. After 20 hours at 20°C. and 60 per cent. R.H., the larva moults. It moults three times more before pupating, the second, third and fourth instars each lasting approximately 24 hours at 20°C. and 60 per cent. R.H.

There does not appear to be any change in the feeding habits of the parasite during the various larval instars. Each instar remains inside the host cocoon.

When the final (5th) instar is fully fed it pushes the remains of the host and its own exuviae out of the cocoon and then commences to spin its own cocoon. The larva remains inside the host cocoon which is incorporated in the cocoon of the parasite. *Ephialtes* thus uses the *Plutella* cocoon as a framework for its own. The finished cocoon is similar to that of *Plutella* except that the mesh is now extremely dense and fine and the shape is slightly different. Approximately two days are spent on spinning the cocoon, the fifth instar thus lasting about three days. After the cocoon has been spun the larva becomes quiescent and pupates. The pupal period lasts between six and seven days at 20°C. and 60 per cent. R.H.

The adult insect emerges from the cocoon by gnawing through one end. The entire life-cycle from egg to adult is completed in 16 days at 20°C. and 60 per cent. R.H.

Description of the Developmental Stages.

Nomenclature for the parts of the cephalic skeleton.

Thorpe (1930), Salt (1931), Vance & Smith (1933), Cameron (1938), Beirne (1941) and Short (1952) have described the larvae of various parasitic Hymenoptera. The nomenclature they use for the different parts of the cephalic skeleton, however, varies. Thorpe, Salt and Cameron, have used systems based on the probable function of the cephalic larval structures and they imply no homology with adult structures.

The systems used by Vance & Smith (1933), Beirne (1941) and Short (1952), on the other hand, are based on an attempt to homologue the adult tentorial structures with the sclerotised rods of the larva. Vance & Smith (1933) rejected Thorpe's nomenclature on the grounds that there were "more elegant and technical terms". These technical terms, however, were terms used to describe the adult tentorium. Beirne (1941) rejects Thorpe's system because it does not imply homology and in fact uses Thorpe's statement (Thorpe, 1930, p. 404) "The use of these names does not necessarily imply any homology with the structures found in the adult" to condemn Thorpe's system. Short (1952) quite definitely means his terms to imply homology.

Observations during the present study agree with Salt (1931, p. 501) who states that "the sclerotised rods in the head of the larva do not seem to be

wholly cuticular nor are they internal ridges corresponding to external sutures" (*i.e.*, as in the adult tentorium of the generalised adult insect). It would thus seem that they are different structures from those of the adult. Again Beirne (1941, p. 123) places the sclerotised rods of the larval Ichneumonid head in two categories, namely "the external cephalic skeleton" and "an internal system of struts known as the tentorium". He again points this out (p. 129) and at the same time states "Thorpe (1930) uses the term tentorium to designate the external structures as well as the internal, but as Vance and Smith (1933) pointed out this is not accurate. The word tentorium should be used for the internal skeleton only". Now if this is so, it is hard to realise why such terms as epistoma, hypostoma, and pleurostoma, which relate to the internal tentorium of the adult insect, should be applied to what Beirne considers external sclerites.

It was thus decided to use a system based on Thorpe with modifications due to following the function of the structures through the five instars. No homology with adult structures is implied in the nomenclature in this paper though structures which are considered to be homologous in the several instars are given the same name. The relation between the terminology used in this paper and the terminologies of Short and Beirne is shown below:—

Short.	Beirne.	Present author.
hypostoma	hypostoma	basal arch (bas. a.)
epistoma	epistoma	clypeal arch (clyp. a.)
posterior pleurostomal ramus	inferior pleurostomal ramus	inferior mandibular strut (inf. md. st.)
labial sclerite	labial sclerome	{ labial plate (lab. pl.) labial ring (lab. r.)
labral sclerite	labral sclerome	labral arch (lbr. a.)
anterior tentorial arm	anterior tentorial ramus	longitudinal posterior strut (l. p. st.)
sclerotic spur of hypostoma	stipital sclerome	maxillary arch (max. a.)
pleurostoma	pleurostoma	mandibular arch (md. a.)
anterior pleurostomal process	superior pleurostomal ramus	superior mandibular strut (sup. md. st.)
tentorial bar	transverse tentorial bar	transverse posterior strut (tr. p. st.)

The egg.

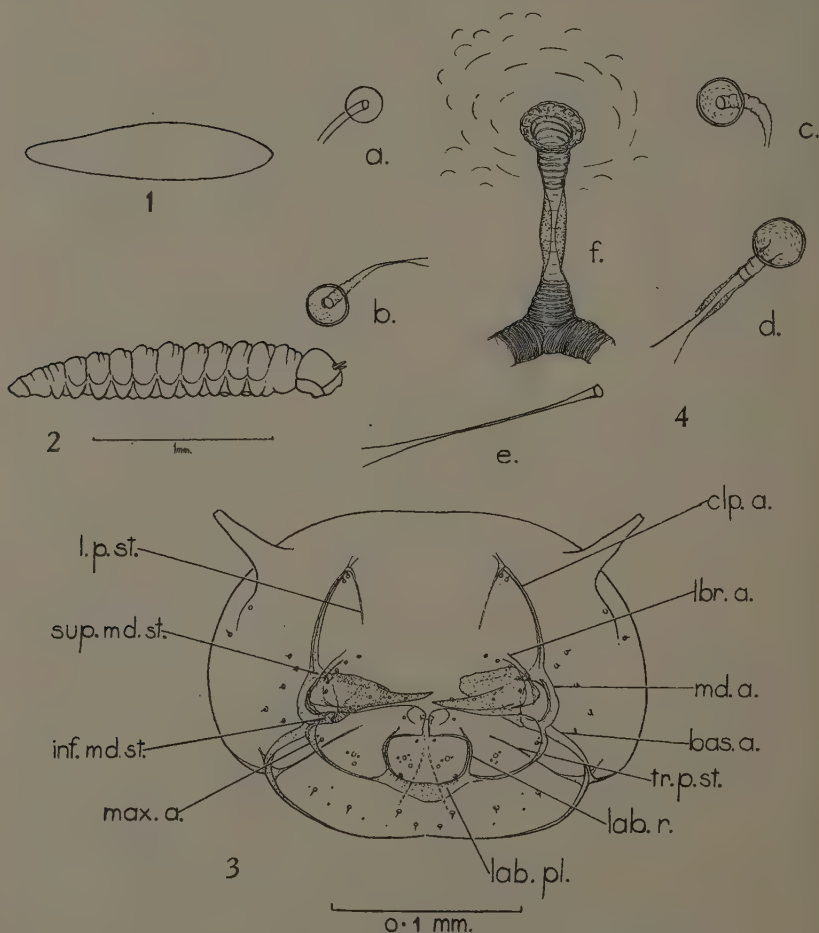
The egg (fig. 1) is whitish in colour, shiny and one end is slightly broader than the other. The average length is approximately 1.4 mm. and the maximum breadth is 0.3 mm.

The larval instars.

All five instars (figs. 2, 5, 7, 9, 11) are of the characteristic external Ichneumonid type. All are white or whitish-yellow, fusiform (except for the first instar which tapers from the head posteriorly) and all have 13 body segments. In the first instar there are dorsal folds on all segments, paired lateral pleural lobes on thoracic segments 2 and 3 and abdominal segments 1-7, and ventro-lateral lobes, which apparently have a locomotory function, on thoracic segments 2 and 3 and abdominal segments 1-8. The second instar is similar to the first but the dorsal folds are less prominent. In the third instar there are no dorsal folds, the ventral lobes are smaller in relation to the size of the whole larva, and the anal segment is transversely grooved. In the fourth instar, dorsal welts or ridges appear, the ventral lobes are more reduced though the pleural lobes are prominent and the anal segment is deeply transversely grooved. In the final instar there are prominent dorsal welts on the first seven abdominal segments, paired pleural and ventral lobes on thoracic segments 2 and 3, and on the first 8 abdominal ones; the anal segment is very much transversely grooved. The lengths of the instars are roughly: first 1.8 mm., second 1.9 mm., third 2.8 mm., fourth 3.7 mm., fifth 5.0 mm.

The integument, from being comparatively smooth in the first instar with the tuberculae occurring in bands on all segments and being more developed on the last one, becomes increasingly more tuberculate and setose, and in the final instar it is very densely tuberculate with prominent setae (fig. 12).

The head capsule becomes increasingly more sclerotised until in the final instar it is quite heavily sclerotised. Tuberculae appear in the fourth instar in the regions of the antennae and clypeus and become denser in the fifth instar.



Figs. 1-4.—*Ephialtes brevicornis*. (1) egg; (2) first-instar larva; (3) head capsule of first-instar larva showing cephalic skeleton (front view): (clp. a. = clypeal arch; lbr. a. = labral arch; md. a. = mandibular arch; bas. a. = basal arch; tr. p. st. = transverse posterior strut; lab. r. = labial ring; lab. pl. = labial plate; max. a. = maxillary arch; inf. md. st. = inferior mandibular strut; sup. md. st. = superior mandibular strut; l. p. st. = longitudinal posterior strut); (4) spiracles of various instars: a, first; b, second; c, third; d, e, fourth; f, fifth.

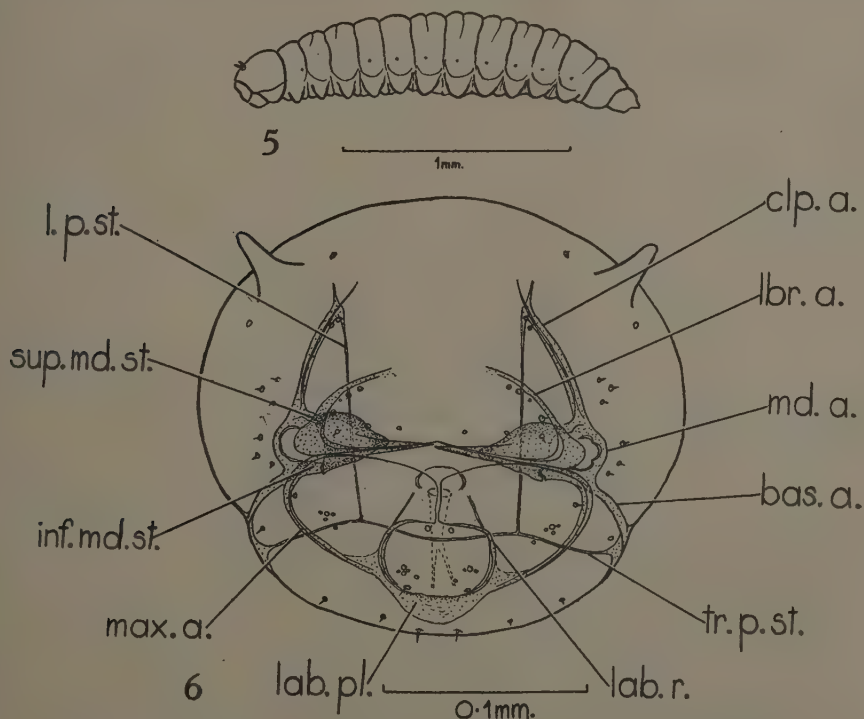
Widths of the head capsule in the various instars are: first instar, 0.275 mm.; second, 0.30 mm.; third, 0.36 mm.; fourth, 0.44 mm.; fifth, 0.60 mm.

The antennae in the first instar are very well developed, large in comparison with the head, not quite conical, and narrow abruptly towards their apices which are somewhat flattened.

In all the other instars the antennae are conical and become smaller and smaller in relation to the head the later the instar. Their bases are never covered with tuberculae and are most developed in the fourth and fifth instars. The approximate lengths of the antennae are: first instar, 0.05 mm.; second instar, 0.039 mm.; third instar, 0.049 mm.; fourth instar, 0.05 mm.; fifth instar, 0.037 mm.

A well-defined salivary duct can be seen in the first two instars opening just below the apices of the mandibles, largely covered by the maxillary lobes and bifurcating posteriorly. In the third instar the anterior portion of the duct is very much wider in relation to the posterior branches than in the earlier instars. In the fourth instar preparations, this anterior portion appears more pouch-like, the posterior branches are no longer visible; and in the fifth instar the duct is finally reduced to a pouch with a sclerotised opening which probably functions as a spinneret.

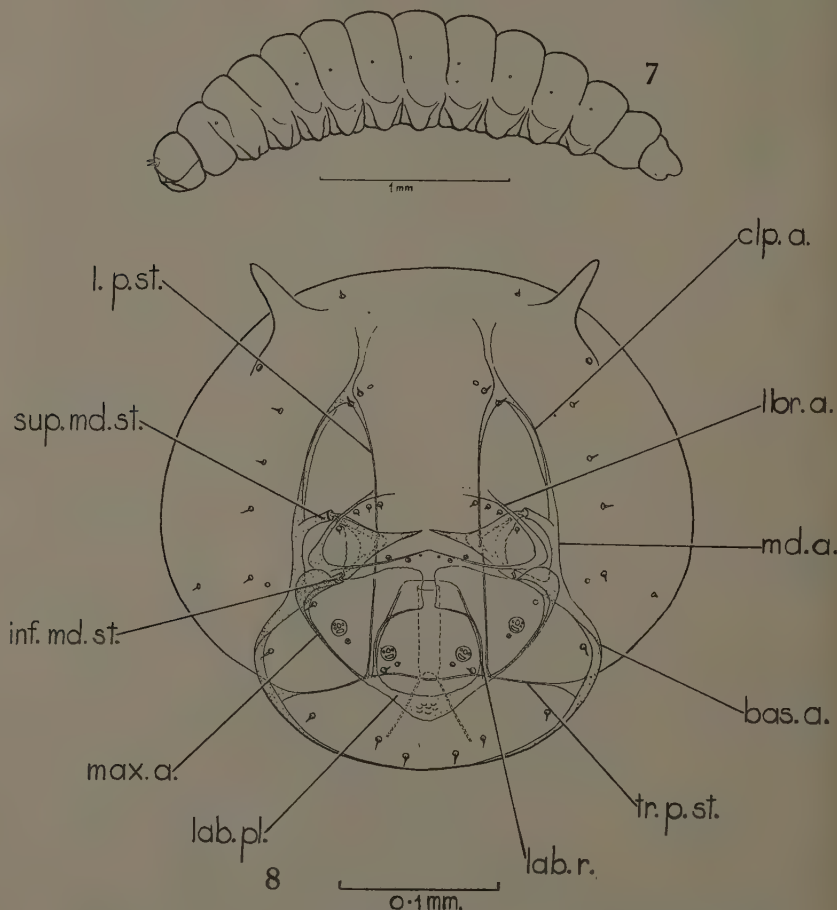
The mandibles in all instars are simple and pointed, articulating above with a condyle on the superior mandibular strut and ventrally with the inferior mandibular strut. Serrations appear in the second instar and become increasingly



Figs. 5-6.—*E. brevicornis*. (5) second-instar larva; (6) head capsule of second-instar larva, showing cephalic skeleton (front view): (lettering as in fig. 3).

prominent in the later ones. Thorpe (1930) has indicated the mandibles of the fifth instar as bearing two rows of bristle-like hairs but it would appear that he has misinterpreted the serrations. The distances between the lower mandible bases in the various instars are: first, 0.1 mm.; second, 0.11 mm.; third, 0.12 mm.; fourth, 0.12 mm.; fifth, 0.145 mm.

The labial area of all instars is bilobed, colourless, and has a paired group of papillae with a prominent seta below each group on it. The area is surrounded and supported by the labial ring. The labial papillae become more definitely grouped in the later instars and in the final instar the main elements appear as paired simple large papillae invaginated distally and similar to the maxillary elements.

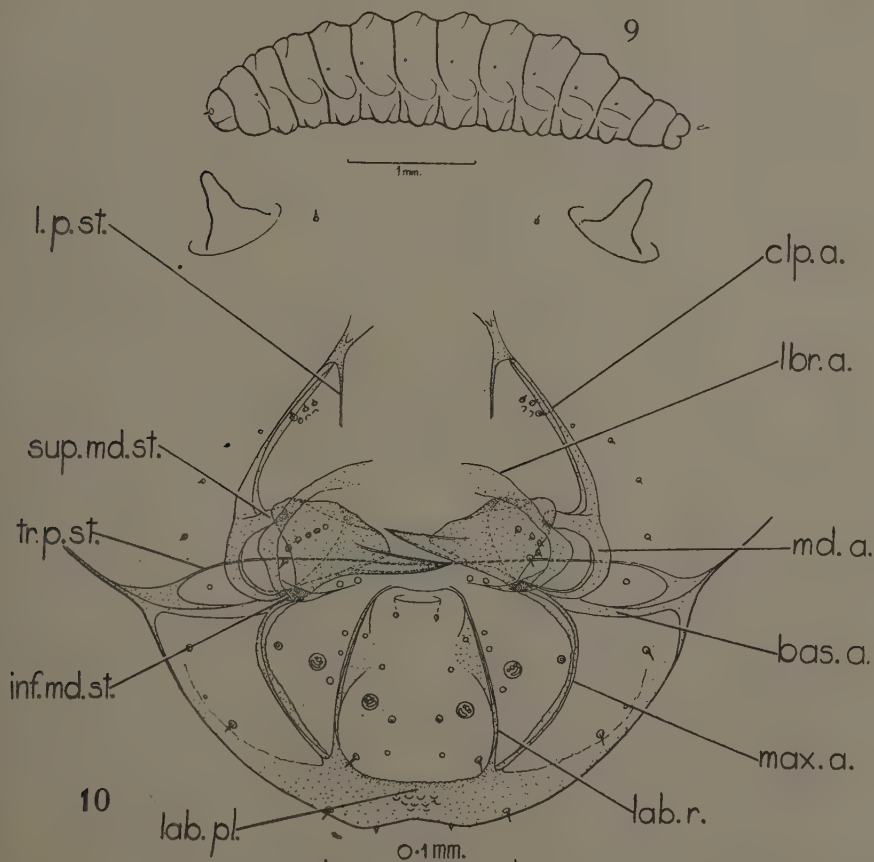


Figs. 7-8.—*E. brevicornis*. (7) third-instar larva; (8) head capsule of third-instar larva, showing cephalic skeleton (front view): (lettering as in fig. 3).

In the first two instars (figs. 3 & 6) a paired group of papillae that perhaps represent the maxillae are situated on colourless, slightly raised areas below the mandibles. These areas are produced into lobes covering the opening of the salivary duct and superior to the labial area. In the third instar (fig. 8) the lobes do not approach each other so closely and the papillae are more definitely grouped, and in the fourth instar (fig. 10) the lobes are very much reduced. In the fifth instar (fig. 13) the lobes have disappeared completely, and the maxillary area is distinctly contoured, its elements being quite similar to the labial ones.

Setae on the head are arranged in groups and surround the main structures of the cephalic skeleton. The numbers, size and position of some groups change in the various instars. The positions of the setae are shown in figs. 3, 6, 8, 10 and 13.

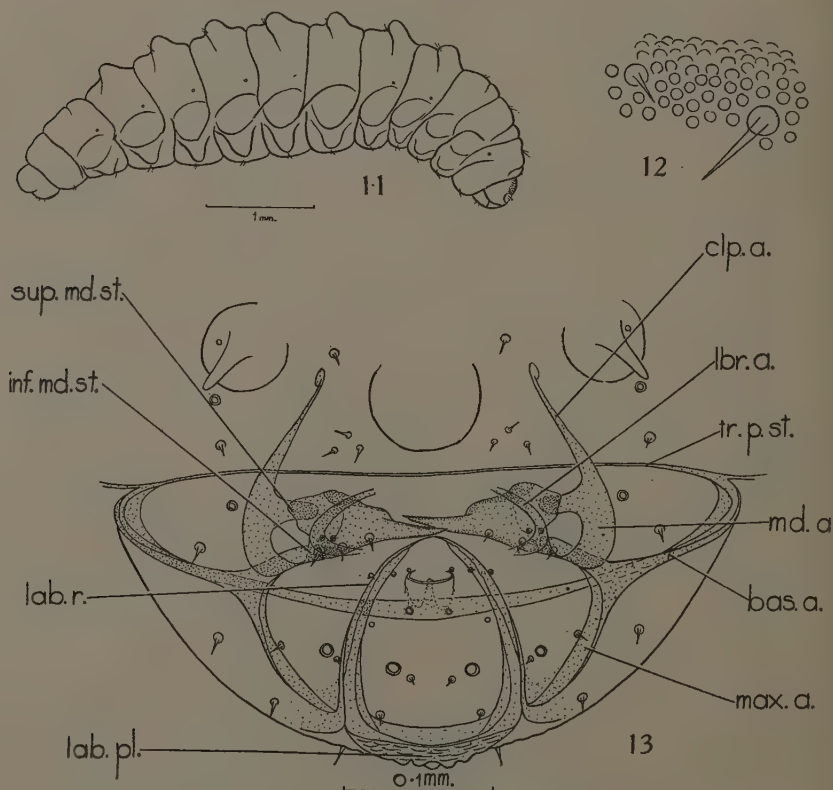
The cephalic skeleton shows changes in each instar. In the first instar (fig. 3) there is a well-defined mandibular arch from which struts articulate with



Figs. 9-10.—*E. brevicornis*. (9) fourth-instar larva; (10) cephalic skeleton of fourth-instar larva (front view): (lettering as in fig. 3).

the mandible bases. An upper arm, from the mandibular arch to the clypeal arch, supports the clypeus. There are two major arches ventral to the mandibles: the maxillary arch which supports the maxillary structures, and the basal arch. These arches fuse with the mandibular arch to form the lower mandibular strut. The labial ring is composed of three main parts: a ventral and anterior horseshoe-like element whose base fuses with a lightly sclerotised labial plate, a pair of elements produced backwards from the horseshoe element, and a more dorsal and posterior inverted crescent-shaped element. These elements in later instars are completely fused and thickened and the dorsal transverse portion of the horseshoe element which supports the maxillary lobes is lost. Among the minor elements of the cephalic skeleton are the beginning of the transverse and longitudinal posterior struts and the labral arch.

In the second instar, all structures are more strongly developed and more easily seen. The elements of the labial ring are similar to those of the previous instar but the labial plate now shows signs of tubercles. The longitudinal and transverse posterior struts are now more prominent and can be seen to join.



Figs. 11-13.—*E. brevicornis*. (11) final-instar larva; (12) portion of integument of final-instar larva; (13) cephalic skeleton of final-instar larva (lettering as in fig. 3).

The arches and struts are more strongly developed and their relative proportions have changed in the third instar. The labial plate is distinctly tuberculate.

Changes in the fourth instar are mainly connected with the reduction of the maxillary lobes and the fact that the mouth-parts are now very much more ventrally placed. The maxillary and clypeal arches are strongly developed while strong sclerotisation of the cuticle is more widespread. The labial ring has now lost the transverse elements which supported the lower margins of the maxillary lobes and it now surrounds both the labial area and the opening of the salivary duct. The labial plate is larger and the tubercles on its surface have become stronger and more pronounced. The basal arch now curves more upwards and the transverse posterior strut is more dorsally placed. The connection with the longitudinal posterior struts seems to have disappeared, though the dorsal portion of these struts can be made out.

In the fifth instar, most structures are very much thickened and the longitudinal transverse struts have been lost. The labial ring is now a very solid structure and surrounds the labial elements and the spinneret. The labial plate, which is fused to the labial ring, bears five or six extremely large tubercles on its surface. The basal arch curves distinctly upwards and continues behind the mandibles as the thin transverse posterior strut. The clypeal arches are much reduced in relation to the other structures and emerge on the surface as pits just beside the antennae.

Nine pairs of spiracles are evident in all instars and a very small tenth one (fig. 4, e) was noticed between the second and third thoracic segments of the fourth instar. The paired spiracles occur on the posterior margin of the first thoracic segment and on the anterior margins of abdominal segments 1-8 in the first two instars. In later instars the first spiracle is situated between thoracic segments 1 and 2. In the first and second instars, the spiracles are minute and relatively simple, but they become larger and more complicated in later instars. In the third instar (fig. 4, b), annulation and thickening of the attached tracheid is becoming more evident and in the fourth instar the tracheid is distinctly annulate, and two granular bodies enclosing a narrow channel, and which probably function as a valvular apparatus, follow from its first portion. In the fifth instar the granular bodies are more conspicuous and are approximately 0.03 mm. long. The large atrium in this instar is distinctly sculptured (fig. 4, f) and the trachea bifurcates after a short distance (approximately 0.05 mm.). The approximate atrial diameters of the various instars are: first, 0.011 mm.; second, 0.013 mm.; third, 0.016 mm.; fourth, 0.021 mm.; fifth, 0.029 mm.

Pupa and cocoon.

The pupa is of the typical Ichneumonid form and has much the same characteristics as the adult.

The cocoon is approximately 6 mm. in length and is cylindrical in shape with one end slightly attenuated.

Summary.

Ephialtes brevicornis (Grav.) has been found to parasitise the prepupal stage of *Plutella maculipennis* (Curt.) under laboratory conditions.

A general description of the life-cycle of *E. brevicornis* on *P. maculipennis* is given and it is found to occupy 16 days from egg to adult at 20°C. and a R.H. of 60 per cent. Comparative descriptions are given of the five larval instars. The egg is also described.

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ESTIMATION OF HUMIDITY WITH COBALT THIOCYANATE PAPERS AND PERMANENT COLOUR STANDARDS.

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(PLATE XI.)

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In biological work, both in field and laboratory, it is often desirable to have a measure of the atmospheric humidity in small spaces or crevices. Frequently the standard methods are unsuitable because they involve too much disturbance of the conditions that are to be measured. In such circumstances, the colorimetric estimation of humidity by means of cobalt thiocyanate papers could often provide a convenient way around the difficulties. At the Pest Infestation Laboratory, this paper has proved useful for the determination of humidity in small experimental vessels, in crevices, and against surfaces which may not be in moisture equilibrium with the atmosphere, *e.g.*, concrete floors. In humidity gradient apparatus or choice chambers for work on animal behaviour, a strip or series of pieces of the paper has provided a useful picture of the humidity pattern. We have also used cobalt thiocyanate paper for the measurement of humidity in the atmosphere within masses of stored grain, etc., sometimes as an alternative to the determination of the water content of the material; for the latter purpose, a sample may be enclosed in a tube or bottle with an impervious stopper, together with a piece of the paper, which can be extracted in due course for examination. Alternatively, the colour of the paper may be examined, less effectively, through the glass.

Over fifty years ago, Wetherell (1905) described the use of filter paper impregnated with cobalt chloride as an indicator of sweating from the human skin. Darrow (1943) added various amounts of glycerin to give a graded series of papers for this purpose. The same salt in collodion was used to measure the cuticular transpiration of insects (Eder, 1940). But cobalt chloride paper is probably best

known as an indicator of transpiration from leaves (Livingstone & Shreve, 1916; Henderson, 1936).

The first attempt to use cobalt chloride paper in the quantitative estimation of humidity was probably that of Atkinson (1923), who proposed the use of printed colours as standards. This was unknown to me when (Solomon, 1945) I described the use of cobalt chloride paper for measuring the humidity of the atmosphere and of the air spaces of solid materials. I reported preliminary experiments in which humidity papers of various properties were made by using cobalt chloride with other salts and on other supports than paper, and an account was given of the preparation and use of greatly improved humidity papers made with cobalt thiocyanate. Two further new developments were introduced to make more accurate measurements possible: the immersion of the test papers in oil for examination, and the use of colour standards made by exposing papers to known humidities and mounting them in oil. This paper also dealt briefly with some aspects of the physico-chemical background of the method.

In the U.S.A., several versions are available of a humidity-indicator card with 6 or more spots of cobalt chloride in graded concentrations, probably mixed with other salts. The number of spots showing a blue colour gives a rough indication of the humidity. Specifications of mixtures for use as indicators have been given by Chatt & others (1948), Davis & Pryor (1950) and by Goodwin & Simpson (1953).

Pomeranz & Lindner (1953) have used cobalt chloride paper for humidity measurement as an indirect index of the moisture content of milled products. Cobalt chloride with phosphoric acid on silica gel has been recommended by Horita (1954) for the estimation of moisture in powdered chemicals. Ward & Tischer (1953) applied cobalt chloride in alcoholic solution to partly dried corn kernels to show the distribution of moisture.

Some of the methods and materials described in the earlier paper (Solomon, 1945) have been taken up by biologists engaged in field work (*e.g.*, Davies (1948), Cragg (1950), Delany (1953), Macfadyen (1953), Morton (1954), Broadhead & Thornton (1955)), and cobalt thiocyanate papers were used for some years by the staff of the Infestation Control Division of the Ministry of Agriculture, Fisheries & Food, until the maintenance of paper-in-oil standards became too great a burden.

In spite of this difficulty, the methods described in the earlier paper may have advantages for certain special purposes: the maximum accuracy can be achieved only by the use of standards made from the same batch of paper as is used in the determinations and recently exposed over constant humidity solutions at a steady temperature; and it is an advantage if the whole of the work can be done at one temperature. But the preparation of standards in this way is relatively troublesome, and for general purposes the use of ready-made impregnated paper and unvarying coloured glass standards is very much more convenient.

The present paper gives an account of the use of cobalt thiocyanate paper with coloured glass standards, both of which are now commercially available. This method cannot replace standard techniques, such as the dew-point, when rapid or very accurate determinations are required, but for the special purposes mentioned above, and often for general use when an approximate estimate is sufficient, it has proved to be very convenient.

Account of the Method.

Summarily, the procedure is to expose a small piece of the impregnated tissue paper, preferably for two hours, at the site where the humidity is to be estimated, then immerse it in liquid paraffin, mount it doubled over in a small quantity of

the oil on a piece of white opal glass, cover it with clear glass, and finally to match the colour by reflected light against coloured glasses, labelled with the relative humidities corresponding to the colours, in a simple comparator. (Pl. XI, figs. 3 & 4.)

The paper.

The paper is a thin cellulose "condenser tissue". Several methods of impregnation were described in the earlier paper (Solomon, 1945), but since impregnated paper is now readily available, these will not be repeated. The amount of salt in the paper has been standardised at 0.55 mg. anhydrous $\text{Co}(\text{CNS})_2$ per sq. cm.

The stock of impregnated paper should not be exposed to extreme temperatures or humidities. As will be shown in a later section, there is some advantage in keeping the paper, at least for a time before it is used, in a desiccator or elsewhere at a relative humidity of about 60–70 per cent. To avoid contamination, clean forceps and scissors should be used in cutting it up. The salt is very soluble in water and the common solvents, so that the paper becomes blotchy if wetted. It may be permanently discoloured by chemical vapours, such as ammonia. When a piece is being exposed, it should be protected from excessive dust; flour has been found to produce a discoloration of the paper.

The size of the pieces used as test papers may be varied at convenience; a piece 1.5×3 cm. is generally suitable, and just fills the field in the comparator, when doubled over.

Immersing and mounting.

At the end of the period of exposure, a test paper should be immersed in a dish or tube of liquid paraffin (Pl. XI, fig. 2) either at the site of exposure or immediately upon removal, to prevent any loss or gain of moisture from exposure to another humidity. (If there is unavoidable delay between removal and immersion of the paper, it may be sufficiently protected for a short time by sandwiching between two pieces of glass, wider than the paper, or by folding it between thin layers of a non-absorbent material such as polythene.) The liquid paraffin used should be the laboratory reagent in preference to the technical or medicinal grades, which tend to deteriorate after long storage.

In preparation for matching, a test paper should be lifted from the oil, placed on a piece of white opal glass, doubled over, and covered with a piece of clear glass (Pl. XI, fig. 3). Usually, enough oil will have been transferred with the paper to fill the space which should be left between the edges of the paper and the edges of the cover glass, thus sealing it off from the outside atmosphere; more oil may easily be added if necessary. For pieces of paper which fold over to a size of about 1.5×1.5 cm., a convenient size for the white glass is 2.5×2.5 cm., with a colourless cover glass slightly smaller. These pieces of glass may be cleaned before use by washing in hot water and drying with a clean cloth.

Once under the oil, whether mounted or loose in a tube, a test paper may be kept for hours or even a few days before matching. If the waiting period is much longer than this, or if the relative humidity being measured is below 20 per cent., changes in colour may occur (see p. 500). During the waiting period the papers should not be subjected unnecessarily to extremes of temperature, as for example by being left in direct sunlight.

Coloured glass standards.

The glass standards have been carefully selected by reference to papers exposed at the specified series of humidities over solutions of potassium hydroxide (or sulphuric acid for the lowest humidities), made up to densities listed elsewhere

(Solomon, 1951). These solutions were kept at a constant temperature of 20°C. in reagent bottles which were somewhat less than half full, and closed by means of rubber stoppers. A pin was pushed into the underside of each stopper, and bent to form a hook, from which the paper could be suspended over the solution by means of a thin wire with a loop at the upper end (Pl. XI, fig. 1) and later removed as shown in Plate XI, fig. 2.

The coloured glasses are labelled with relative humidity values in steps of 5 per cent., from 100 down to 60 per cent., and thence in steps of 10 per cent. down to zero. They are mounted in two plastic discs, either of which may be fitted into the comparator by opening the hinged lid.

Matching.

The test paper, mounted between its white glass base and clear cover glass, is placed on the floor of the comparator stand, vertically in line with the right-hand viewing aperture above (Pl. XI, fig. 4). Beside it on the left, vertically below the left-hand viewing aperture, is placed a white block of magnesium carbonate. Slips of wood are placed under the base-glass of the paper, to raise the paper to about the same level as the white block. Viewed from above, the paper is now visible by reflected light, while the coloured glasses are visible, one at a time as the disc is rotated by its projecting edge on the right, in the left-hand aperture, by the light reflected from the white block. The disc is cut away at the centre to leave the right-hand viewing aperture clear.

For correct lighting, the comparator should be illuminated in front by daylight (not direct sunlight), preferably from the north (in the northern hemisphere). If artificial light is used, the results will lose accuracy to a variable extent, depending on the nature of the light.

Since the colours of the paper vary somewhat with temperature, the matching should be done if possible with the papers at approximately 20°C., the temperature of calibration of the glass standards. The temperature should in any case be noted, and if it differs appreciably from 20°, allowance should be made for this as set out in Tables IV and V.

In matching a paper against the coloured glasses, the aim should be to assess the redness and blueness of the paper relative to that of the glasses. For example, a paper may be a little redder and less blue than the 70 per cent. R.H. glass, and a good deal bluer and less red than the 75 per cent. glass. It might accordingly be given a value such as 71 per cent.

It is desirable to make two independent matchings, of which the mean can be taken, and if they differ by more than 1 per cent., a third reading should be added, and even a fourth if necessary, until one is confident of having arrived at a result free of substantial random error. When there is a number of papers to be matched, it is best to examine them once each in succession, then a second time in the reverse order, and again if there are appreciable inconsistencies.

Estimates of Errors.

The task of distinguishing the different sorts of error and estimating their size proved to be difficult. There are some sources of error whose effects intermingle and present problems of disentanglement. An exhaustive analysis would be a much more elaborate project than the present one, which aims to make approximate estimates of the different errors as a guide in the practical use of the method.

Since it has not always been possible to exclude all types of variation other than the one being measured, the results tend to be over-estimates.

For practical guidance, the measure of 95 per cent. confidence limits is one of the most useful types of statistics. These have been calculated by a rough and time-saving method from the observations on variability. First, the standard

deviation was estimated approximately from the observed range of variation, using a table of mean values of the ratio, range/σ (Snedecor, 1946). This estimate was then multiplied by the appropriate figure from a table of values of t , to give 95 per cent. confidence limits. For an estimate of the combined variation from two or more sources (Table VIII), the standard deviations were squared to give variances, which could be added. In selecting the value of t for calculation of the confidence limits in Table VIII, a median number of 10 degrees of freedom was adopted.

Variation of readings by a single observer.

Success in matching the papers accurately against the coloured glasses depends partly on close observation of the colours and on skill in making correct interpolations between successive disc values. Probably one improves with practice.

By the replication of matchings, as recommended above, the inconsistency can be reduced to about ± 0.5 per cent. for relative humidities of 60–100 per cent. and about ± 1 per cent. for lower humidities, when the humidities are close to those represented in the standard colours, and about twice these figures when interpolation is necessary.

Variation between different observers.

When the colours of the impregnated paper are copied in glass, a different set of primary colours is used in the latter. Under these conditions it is impossible to arrive at matchings which appear perfect to all observers, for individuals differ from each other in their relative sensitivity to the colours involved.

It is necessary to enquire how great a range of variation between individuals may be expected. To provide information on this point, 12 members of the staff of the Pest Infestation Laboratory matched six mounted papers which had been exposed to a wide range of humidities. Each observer matched the papers in order of blueness or redness, then repeated the matchings in the reverse order; the means of the pairs of readings were used in the subsequent calculations. All the matchings were done by daylight, at 20°C. This experiment was spread over three days, during which time the writer matched the papers daily and confirmed that no appreciable changes had occurred.

On the final day, a similar set of six papers covering a wide humidity range was provided for a second matching test by the nine observers (including the writer) who were available on that day. Meanwhile, four of these observers had also repeated the test with the first series of papers.

For each test, the mean value attributed by each observer to each paper was entered on a graph sheet graduated in percentage R.H. Taking as the reference point for each paper the median value attributed, the other results were then read off as deviations above or below this value. These deviations are shown in Table I. The humidities given at the heads of the columns are approximations to the median values. Where an observer took part in more than one test, mean values of the deviations at each humidity level are given in italics below the separate deviations, and in the right hand column of the Table the grand means of deviations for each observer for all humidities are given.

A number of points emerges from this Table:—

1. Not only do observers differ among themselves in the values they attribute to a paper, but there is a certain consistency in the trend of the deviations of most individuals indicated in a rough way by the grand means in the right-hand column.
2. An individual trend may be reversed at some level in the humidity scale (*cf.*, EMR, JMP, AMC).

TABLE I.

Variation between estimates by 13 observers of papers at 6 different humidity levels.

Observer	Test	Deviation from median at R.H. % approx.						Algebraic mean deviation
		88	82	74	63	48	24	
JMP ..	(1)	-2	0	1.5	+3	+3.5	+6	+2.0
	(2)	-1.5	0	-0.5	+1.5	+6	+7	
	Mean	-1.75	0	+0.5	+2.25	+4.75	+6.5	
GAH ..	(1)	0	0	+1	+2.5	0	+2.5	+1.5
	(2)	-0.5	+0.5	0	+3.5	+4.5	+4	
	Mean	-0.25	+0.25	+0.5	+3.0	+2.25	+3.25	
EMR ..	(1)	+1.5	+3	+3	+5	0	-9	+1.0
	(2)	0	+0.5	+1	+2	+7	-1.5	
	Mean	+0.75	+1.75	+2.0	+3.5	+3.5	-5.25	
GEW ..	(1)	+1	+4	+2	+4	0	+6	+0.9
	(1a)	0	+2	+1	+1	0	-4	
	(2)	-1	-1.5	0	0	+0.5	+1	
	Mean	0	+1.5	+1.0	+1.7	+0.2	+1.0	
BDH ..	(1)	-3	-1	-2	0	+2.5	+6	+0.4
TAO ..	(1)	-	0	-1	0	0	+1	0
AMC ..	(1)	+1	+1	+1	-1	0	-4.5	-0.4
RWH ..	(1)	+1.5	-0.5	-0.5	+0.5	-8	-4	-0.7
	(2)	+1.5	+0.5	+0.5	0	-2.5	+2.5	
	Mean	+1.5	0	0	+0.25	-5.25	-0.8	
JOB ..	(1)	-1	0	0	-2.5	-1.5	0	-1.0
	(1a)	-1.5	+1	+1.5	+0.5	-2	-5.5	
	(2)	+0.5	+0.5	0	-0.5	-2.5	-4	
	Mean	-0.7	+0.5	+0.5	-0.8	-2.0	-3.2	
CWC ..	(1)	0	+1.5	0	+1	-4	-9	-1.1
	(1a)	-1	+1.5	+2.5	+1	-0.5	-4	
	(2)	0	-1	-2.5	+1	-1	-5	
	Mean	-0.3	+0.7	0	+1.0	-1.8	-6.0	
JHH ..	(1)	0	-1	-2.5	-1	-3.5	+1	-1.2
HDB ..	(1)	-0.5	0	-1	-5	-4.5	-4	-2.1
	(1a)	0	+1	0	-3	-4.5	-7	
	(2)	+0.5	-1.5	-2	-2	-0.5	-2	
	Mean	0	-0.25	-1.0	-3.7	-3.2	-4.3	
MES ..	(1)	-0.5	-1	-2	-7	-7	-4.5	-2.3
	(2)	-0.5	-1.5	-2.5	-1	0	0	
	Mean	-0.5	-1.25	-2.25	-4	-3.5	-2.25	
RANGE :								
13 observers	(1)	4.5	5.5	5.5	12	15	16	
9 "	(1)	4.5	5.0	5.5	12	11.5	15	
same 9	(2)	3.0	2.0	3.5	5.5	9.5	12	

The entries are deviations of individual estimates from the median value, at each humidity level.

3. The deviations tend to be greater at the medium and low humidities, and so do the ranges of variation between individuals, shown in the three bottom lines of the Table.
4. The ranges of variation between observers were reduced in the final test as compared with those in the first test, presumably as a result of practice. (Two lowest rows of Table. Only a few of the observers had matched a few cobalt papers before, and most were entirely unused to it, or to other colorimetric work.)
5. By reference to the lowest row of Table I the figures in Table II have been prepared. Since further practice might be expected to have still further reduced the variation between observers, these figures probably overestimate it.

TABLE II.

The range of variation in the estimates of 9 observers, as indicated by Table I, test (2), and corresponding 95 per cent. confidence limits for one estimation (duplicate matching) by one observer of unknown bias.

Rel. humidity (%)	Range (9 observers)	Variance ($=s^2$)	95% conf. limits
100	3	1.02	± 2.5
95	3	1.02	± 2.5
90	3	1.02	± 2.5
85	2	0.45	± 1.5
80	2	0.45	± 1.5
75	3	1.02	± 2.5
70	4	1.81	± 3
65	5	2.83	± 4
60	6	4.08	± 4.5
50	9	9.18	± 7
40	11	13.72	± 8.5
30	12	16.32	± 9.5
20	12	16.32	± 9.5

Variation in the papers.

Within a single batch of impregnated paper, there is a certain amount of unavoidable variation in the exact colour the paper will assume at a given humidity. There may be some variation between different batches of paper, due to variations in the impregnation, but this is expected to be relatively small. When two batches were compared over almost the entire humidity range using 5 to 8 replicates from each batch at each humidity, the differences between the samples from the two batches were not significant except at the highest relative humidities (90% and over), where the mean difference was about 2 per cent.

In assessing the variation in the paper, samples from these two batches were taken together, so that any differences between the two were treated as part of the general variation, which at most humidities was chiefly within batches. The samples were exposed for two hours in constant humidity bottles at 20°C., then mounted in oil in the usual way and matched at the same temperature against coloured glass standards. The figures in the right-hand column of Table III may be taken as a guide to the degree of inaccuracy from this source. For example, if a paper is matched at 20°C. soon after exposure and given the value 82 per cent. R.H. by comparison with the standards, there is a 19/20 chance that the true humidity was between 80 and 84 per cent., assuming that the standards are correct, the matching reasonably accurate, and the data in Table III sufficiently representative.

TABLE III.

An estimate of variation in cobalt thiocyanate papers, as measured by the range of humidity values when different pieces are exposed to the same humidity and matched against coloured glass standards, all at 20°C.

Exposed at R.H. (%)	No. of papers	Range of readings	Variance (=s ²)	95% conf. limits
100	12	3.5	1.17	±2.5
95	13	4.5	1.82	±3
90	13	3.25	0.95	±2
85	13	2.25	0.46	±1.5
80	13	2.75	0.68	±2
75	13	2.25	0.46	±1.5
70	13	5.0	2.20	±3.5
65	13	4.75	2.03	±3
60	13	7.0	4.41	±4.5
50	13	9.75	7.98	±6.5
40	13	10.25	9.45	±6.5
30	13	13.25	15.80	±8.5
20	13	7.5	5.06	±5

In the above tests, the effects of the previous history of the paper, particularly the conditions at which it was kept prior to exposure, may have had some influence on the results. Some aspects of this sort of variation are dealt with below.

Effects of storing paper under dry conditions.

The papers have generally been stored in cardboard boxes in the laboratory. After storage at moderate relative humidities, such as 50–70 per cent., they behaved well over the whole range of humidities. In winter, when the relative humidity of the room fell to about 30 per cent., the papers tended to give low readings between 20 and 75 per cent. R.H. The errors increased with reduction of the humidity being measured, and at 40 and 30 per cent. they were sometimes as great as 7 per cent.

It was found that this could usually be greatly reduced by exposing the dry paper for a few hours or longer at a higher relative humidity such as 60 or 70 per cent. before use.

The changes occurring in papers stored for various lengths of time under different conditions of humidity and temperature appear to be complex and have not been fully analysed. Probably the best procedure is to store papers in the region of 60 or 70 per cent. R.H. before they are used. For this purpose a controlled-humidity room may be suitable, or else a desiccator containing an appropriate solution of potassium hydroxide, sulphuric acid, or perhaps a saturated solution of an appropriate salt, choosing one which is unlikely to give off any harmful vapour.

Some of the earlier batches of paper developed brown patches, after storage at low humidity, on parts of the paper where the density of impregnation was a little higher than the standard level. The brown deposit was re-sorbed, and the paper was again suitable for use, after a brief exposure at a higher relative humidity, such as 70 per cent.

Effects of matching-temperature.

The standard colours were determined by reference to papers exposed to a series of humidities at 20°C. When papers are matched at other temperatures, corrections should be applied. A paper becomes bluer and less red as the

temperature rises, giving a lower humidity reading, and contrariwise as the temperature falls. If a paper of a given colour, after immersion in oil at 20°C., is subsequently moved to other temperatures and the colours are read off as humidities, these values form a rectilinear slope on a graph of colour values (as humidities) versus temperatures. In Table IV these slopes are indicated by the mean errors per 10° temperature difference from 20°C. The data are the results of matching numerous mounted papers, each at two or more temperatures within the range -5° to +40°C.

(This set of rectilinear slopes cannot be extrapolated indefinitely beyond the temperatures mentioned, otherwise they would intersect at various points, and this would represent a physical absurdity. Their straightness is no doubt only apparent, and they may presumably be quite strongly curved in other parts of the temperature range.)

TABLE IV.

Effects of matching papers at temperatures other than the calibration temperature (20°C.).

Papers exposed* at R.H. %	Number of examples	Mean R.H. difference per 10°C. (smoothed)
100	12	1.5
95	10	2.5
90	17	3.0
85	18	3.5
80	12	4.0
75	24	4.5
70	12	5.0
65	12	5.5
60	15	6.0
55	8	6.5
50	4	7.0
45	10	6.5
40	7	6.0
35	6	5.5
30	5	5.0
25	6	3.0
20	16	1.0

For each exposure-humidity, these effects are approximately in linear proportion to the temperature-difference. As the temperature rises, a paper in oil becomes bluer and less red, giving a lower humidity reading, with reverse results as temperature falls.

* Most were exposed at 20°C. For the others, it is the humidity value after removal (in oil) to 20°C. which is referred to in this column.

In normal practice, if a paper is matched at say, 30°C., we require to make a correction on the basis of that reading, not on the basis of the exposure-humidity, for the latter is the unknown we are trying to determine. For this purpose, Table V gives a series of corrections, using values derived graphically from those of Table IV.

Effects of changes in temperature.

Contrary to the indications of a test referred to in the earlier paper (Solomon, 1945), no systematic error has since been found in measuring humidities at 10° and 30°C., provided the corrections of Table V are applied. In other words, papers exposed to the same humidity at different temperatures, and matched at those temperatures, appear to give the same humidity values after correction

from Table V, subject to the usual random variations. But if they are brought to another temperature for matching, the variation is increased.

Thus, in plotting the data used in Table IV, there was found to be a considerable scatter of points about the mean slopes. In many cases papers mounted in oil were moved from one temperature to another, and back again, and sometimes to yet another. They were held at each temperature long enough for

TABLE V.

Corrections to be made when papers are matched at temperatures other than 20°C.

Approx. R.H. indicated by paper	Temperature (°C.)							
	40°	35°	30°	25°	15°	10°	5°	0°
103	—	—	—	—	—	—	—	— 3
100	—	—	—	—	—1	—2	— 3	— 5
95	+ 3.5	+ 3	+2	+1	—1.5	—3	— 4.5	— 6
90	+ 5	+ 4	+2.5	+1.5	—1.5	—3.5	— 5.5	— 7.5
85	+ 6	+ 4.5	+3	+1.5	—2	—4	— 6	— 9
80	+ 6.5	+ 5	+3.5	+2	—2	—4.5	— 7	—10
75	+ 7.5	+ 6	+4	+2	—2.5	—5	— 8	—11
70	+ 8.5	+ 6.5	+4.5	+2.5	—2.5	—5.5	— 8	—12.5
65	+ 9	+ 7	+5	+2.5	—3	—6	— 9.5	—13.5
60	+10	+ 8	+5.5	+3	—3	—6.5	—10.5	—13.5
55	+11	+ 8.5	+6	+3	—3.5	—7	—10	—12.5
50	+11.5	+ 9	+6.5	+3.5	—3.5	—6.5	— 9	—11.5
45	+12.5	+10	+7	+3.5	—3	—6	— 8.5	—11
40	+13.5	+10.5	+6.5	+3	—3	—5.5	— 8	—10
35	+14	+10	+6	+3	—2.5	—5	— 6.5	— 8
30	+12.5	+ 9	+5.5	+2.5	—2	—3.5	— 4.5	— 5.5
25	+11	+ 8	+5	+2	—1.5	—2	— 3	— 3.5
20	+10	+ 3.5	+1.5	+0.5				— 4
18	+ 2							

To exemplify the use of the Table: if a paper reads 75 per cent. R.H. at 35°C., the corrected value is $75 + 6 = 81$ per cent.

temperature equilibrium to be established, and then matched against the glass standards before removal to warmer or cooler conditions. Often a paper would give a somewhat different colour at 20°C., after being taken, for example, to 30°C. and returned. Since this sort of variation did not seem to follow any recognisable pattern, it could only be attributed to a random disturbance of the blue/red equilibrium, caused by exposing the papers to a change in temperature. To give a measure of this variation, the standard deviation and 95 per cent. confidence limits were estimated for each level of humidity, by means of the method described on p. 492, the range being taken as twice the maximum deviation of the observations from the temperature-correction slope for that humidity. The values are given in Table VI. The grouping of data for different humidities was adopted because examination of the graphs suggested that there were no real differences in variability between the sets of data concerned.

In Table VI the standard deviations refer to the whole series of points over the range of temperature studied, and do not increase or decrease in any regular way with the amount of temperature difference from 20°C. Some of the greatest deviations from the linear slope were at extreme temperatures such as 40° or -2°C., but at most humidities this tendency was not apparent. Hence, for a paper of given moisture content, the confidence limits are taken to be the same,

whether one is making a small correction to a reading made at 25°, or a correction 4 times as great to a reading made at 40°C.

The application of Tables V and VI may be summarised as follows:—

When papers are exposed at one temperature and matched at another, both the matching-temperature corrections should be applied (Table V).

TABLE VI.

Variation of papers in oil under disturbing influence of removal from one temperature to another.

% R.H. value at 20°C.	No. of examples	Range=2 × max. deviation	Variance	95% conf. limits
100 to 70	105	6	1.41	±2.5
65 to 25	73	16	11.11	±6.5
20	16	9	6.50	±5.5

The range values are derived from the deviations of values of individual papers from the mean temperature slopes of Table IV. The above confidence limits apply to the variation caused by exposing a paper at one temperature and matching at a substantially different temperature.

When papers are matched at 20°C., after exposure at a different temperature, the confidence limits of Table VI apply.

When papers are exposed at one temperature and matched at another, both different from 20°C., both the corrections and the confidence limits apply (Tables V and VI).

Clearly it is best to expose and match papers at 20°C. when possible; when this is not possible, it is better to match the papers at the exposure-temperature and apply the corrections of Table V than to bring them to another temperature for matching, especially if this latter temperature is not 20°C.

TABLE VII.

Changes in R.H. values, measured as colour by matching, in papers exposed for short periods to humidities different from those at which they had been equilibrated, to test effect of delay in immersion in oil.

Experiment number	1	2	3	4
Paper exposed 2 hours at R.H. (%)	75	65	65	30
Ambient R.H. (%) during transit to oil ..	30	40	47	85
% R.H. change in paper, transit period 5 sec.	2.5	4	(1.75)	3
" " " " " " 10 "	—	6	3.25	—
" " " " " " 15 "	—	(7)	4.5	—
" " " " " " 20 "	—	8	5	—
" " " " " " 30 "	—	10	5.5	—

The figures in brackets were estimated by graphical interpolation.

Changes in colour during transference to oil.

Ideally, test papers should be immersed in oil before removal from the humidity which is to be measured. When this is not possible, a period of delay, during which the paper is exposed to a different humidity, may allow the paper to gain or lose sufficient moisture to affect the colour. The transference to oil normally takes only a few seconds, but, even in such a short time, appreciable changes may occur.

To throw light on this point, a number of papers were equilibrated for two hours at various humidities, then exposed to other humidities for short periods before immersion in oil. These experiments were at 20°C., in a small closed room with little air movement. The results, in Table VII, show how rapidly a change in moisture content, representing several degrees of relative humidity, can take place. They also suggest that the rate of change depends on the degree of difference between the initial humidity and the transit humidity, and that papers from the higher humidities change less rapidly, perhaps because of the greater quantities of moisture required to effect a 1 per cent. change in humidity.

Gradual changes in colour of papers in oil.

Even when immersed in oil without delay, papers that have been exposed at relative humidities below 20 per cent. undergo a progressive change in colour towards lower humidity values which is often detectable within a few hours. After a few days no reliance whatever can be placed in them.

Papers exposed at humidities from 20 per cent. upwards seldom undergo any significant colour change in the first day or two (unless the mounted papers are left exposed to sunlight or otherwise ill-treated). But after 10-15 days there are usually some detectable changes; papers from 100 per cent. R.H. tend to become paler; those from 20-85 per cent. R.H. often give readings 1 or 2 per cent. lower than at first. This latter trend continues slowly, so that papers kept in oil often read 3 per cent. R.H. too low after three months, about 4 per cent. too low after six months, and about 8 per cent. too low after 12 months. This change may be due to a slow moisture loss through the oil into a dry room atmosphere. In any case, it can be retarded by sealing up the edges of a paper-in-oil mount (Solomon, 1945).

For the present purposes, it is clearly preferable to match the papers soon after they have been exposed, although a delay of a day or two usually has little effect.

Effects of short and long exposure periods.

The papers on which the standard colours are based were all exposed for two hours over constant humidity solutions at 20°C. The following data on the effects of shorter or longer exposure periods on the colours of the papers supersede the less accurate figures given in the earlier paper (Solomon, 1945, Table 8).

When a piece of the impregnated paper is newly exposed to a changed humidity, the change in colour is rapid at first, slowing down gradually as the equilibrium humidity value is approached. The shape of the equilibrium curve depends to some extent on the positions of the initial and final humidities on the scale, as well as on the amount of the difference between them.

Exposure for one hour instead of two does not normally give rise to differences of more than 1 per cent. R.H., though somewhat greater errors have been found in papers exposed at the ends of the humidity range (100, and 20 per cent. or a lower R.H.).

Exposure for only 30 minutes gave errors up to 7.5 per cent. at 100 per cent. R.H., and up to 5 per cent. at 20 per cent. R.H., with errors up to about 2.5 per cent. at less extreme humidities.

In other cases equilibration was completed, as far as could be detected by close visual comparisons, within one hour, or 30 minutes, or even shorter periods such as 15 minutes.

Longer exposure periods, up to a few days at least, apparently make no difference to the equilibrium colour except at the ends of the humidity range. At 100 per cent. R.H., there is a continuous uptake of moisture so that some of the solution tends to drip from the paper, with a consequent weakening of the colour. At 20 per cent. R.H. or lower, there tends to be a continuous slow change in colour towards lower humidity values, although the actual ambient humidity remains constant. This is most marked in papers exposed at low relative humidities such as 0-10 per cent., the papers gradually assuming a greenish tinge.

These changes are distinguished from those which may occur in papers after they have been immersed in oil, and which have been dealt with above.

Assessment of Combined Errors.

As a rough working guide, it may be taken that relative humidities from 100 down to 60 per cent. can be estimated with an accuracy of about ± 5 per cent., and lower values to about ± 10 per cent. (occasionally only to ± 15), provided the corrections of Table V are applied when matching is done at a temperature other than 20°C .

Using the data of previous sections, it is possible to make better estimates of the range of the combined error which may be expected under various conditions. However, the precise values of these estimates should not be taken too literally, for it has often been impossible to deal with a single type of variation with the assurance that all other types have been fully excluded; hence there is a tendency to make over-estimates of the different errors.

Not much need be said about the avoidable sources of error. They include gain or loss of moisture by the papers while they are being transferred to oil, the use of short exposure periods, and the effects of storing the papers under very dry conditions. Normally one takes steps to avoid these; when that is not possible, the error involved must depend on the circumstances, as exemplified on earlier pages. The most troublesome of these types of error is the influence of conditions of storage, and perhaps duration of storage, on the behaviour of the paper. While the more extreme effects can be avoided, there is a residue of less obvious effects which it will scarcely be possible to avoid until this matter has been further investigated; for the present, they must be treated as part of the variability of the paper.

As already mentioned, there are several variants of the straightforward use of cobalt papers for measuring humidity, each involving a different combination of unavoidable sources of error:

1. Paper exposed and matched at 20°C .

The sources of variation are the reading errors, including any personal bias, and variation of the papers. A measure of the variation arising from the differences between individuals is given in Table II, together with some of the variation to be expected between independent estimates of the same paper by one individual. Probably there is little error involved if we assume the latter type of variation, discussed on p. 493, to be fully represented in Tables II and III together.

An estimate of variation in the papers is given in Table III. To combine the two sets of variations, we add the variances of Tables II and III, for each humidity separately, and take square roots to arrive at the combined standard deviations. From these, taking an intermediate figure of 10 to represent the

number of degrees of freedom, 95 per cent. confidence limits can be derived by reference to *t*-tables (see Table VIII, VR + VP).

TABLE VIII.

Variation (as estimated 95% confidence limits for determination of humidity) when papers are matched at 20°C., or matched at another temperature and a correction applied.

% R.H. value at 20°C.	VR Variation in readings due to inaccurate matching and individual bias (Table II)	VP Variation in the papers (Table III)	VT Random variation in readings associated with changes in temperature (Table IV)	VR + VP Variation when paper is exposed and matched at same temp.	VR+VP+VT Variation when paper is exposed and matched at different temps.
100	± 2.5	± 2.5	± 2.5	± 3.5	± 4
95	± 2.5	± 3	± 2.5	± 4	± 4.5
90	± 2.5	± 2	± 2.5	± 3	± 4
85	± 1.5	± 1.5	± 2.5	± 2	± 3.5
80	± 1.5	± 2	± 2.5	± 2.5	± 3.5
75	± 2.5	± 1.5	± 2.5	± 2.5	± 4
70	± 3	± 3.5	± 2.5	± 4.5	± 5
65	± 4	± 3	± 6.5	± 5	± 9
60	± 4.5	± 4.5	± 6.5	± 6.5	± 10
50	± 7	± 6.5	± 6.5	± 9	± 12
40	± 8.5	± 6.5	± 6.5	± 11	± 13
30	± 9.5	± 8.5	± 6.5	± 12.5	± 14.5
20	± 9.5	± 5	± 5.5	± 10.5	± 11.5

2. Paper exposed at a temperature above or below 20°C. and matched at the same temperature.

Here the combined VR + VP confidence limits of the preceding paragraph and Table VIII apply as before, but in addition an adjustment of the reading must be made by reference to Table V, to allow for the effect of temperature on the colour of the paper at matching.

3. Paper exposed at another temperature but matched at 20°C.

In this case no matching-temperature correction is needed, but account must be taken of the variation engendered by changes in temperature (Table VI). Since the above-mentioned VR + VP section of Table VIII also applies, it is necessary to combine the variances as outlined above and derive the corresponding confidence limits (VR + VP + VT in Table VIII).

4. Paper exposed at one temperature and matched at another, both different from 20°C.

Here the variation to be allowed for is the same as in the above paragraph, but in addition a matching temperature correction must be applied from Table V.

Special Procedures for Reduction of Errors.

The foregoing account refers to the straightforward use of the paper under various circumstances. Certain precautions to reduce error were envisaged, including protection of the stored paper from damage and extremes of humidity, prompt immersion of test papers in oil at the end of the period of exposure, and duplicate matching of each paper.

Greater accuracy can be achieved by various special measures which are often practicable. Probably the variability of the paper can be reduced by keeping the stock at a constant humidity, or at a humidity varying between narrow limits. The best level for storage purposes is probably about 60 or 70 per cent. R.H. The use of two or more test papers in estimating a humidity will still further reduce the influence of variation of the paper. Similarly, if each paper is matched three or four times, instead of twice, variation due to inaccurate matching is correspondingly reduced. Finally, if an observer can re-calibrate the standard glasses by comparison with several series of papers exposed over constant humidity solutions, he will have removed most of the errors due to his personal idiosyncrasies of colour vision.

It is difficult to estimate the increases in accuracy that can be gained by any one or a combination of these measures. The possible scope can be seen from Tables II and III and p. 492. It may be that some of the variation of Table VI also comes in fact from these sources.

A different approach to the reduction of error is possible if we make measurements of humidity on a comparative basis. Suppose, for example, we wish to estimate the difference between humidity A and humidity B, both unknown. A good deal of the paper variation can be avoided by using pieces cut from adjacent parts of the same batch; apart from reducing the inherent variation in this way, we also ensure having test papers with identical storage histories.

The test papers should if possible be exposed simultaneously and for the same period to the two humidities, then transferred similarly to oil, mounted, and matched several times each. It is probably better to do this at the exposure-temperature, and make corrections from Table V, than to move the papers to 20°C. for matching. If humidities A and B are not very different, the papers should be compared with each other as well as with the standard glasses, to give a double check on the relative values of the papers. It will be appreciated that the observer's personal bias or peculiarities of colour vision enter equally into all the readings, provided that humidities A and B are not very different. The accuracy of this procedure can be further increased by using two or more test papers at each humidity, and by increased replication of the matching. By thus reducing all the main sources of error we can estimate the difference between A and B with a high degree of precision and reliability.

As distinct from this relative assessment of the two humidities, any assignment of absolute values is at once liable to the effects of errors in calibration of the standard glasses (likely to be significant at this level of accuracy), the bias of the observer's colour vision, and of any untypical features in the paper used or in its previous treatment. All these errors can be avoided, and the estimates yet put on an absolute footing, if we have available one or more carefully prepared constant humidity solutions, over which one or more test papers can be exposed for comparison with the others. To escape the necessity of exposing the papers at different temperatures, the solutions should preferably be at the same temperature as the papers at humidities A and B; in any case, they should be at constant temperature. Given papers which have been exposed to accurately known humidities, but otherwise subject to the same conditions as the other test papers, we have the means of making accurate estimates of the absolute values of humidities A and B.

In realising this level of accuracy, we have passed by stages from a method suitable for general and fairly casual use, to one which demands laboratory refinements such as a constant temperature and constant humidity solutions. The method described in the preceding paragraph does not really require coloured glass standards. There is no doubt that for maximum accuracy, both in matching and in other respects, the best way of using cobalt papers is to set up temporary paper-in-oil standards, using papers exposed over constant humidity solutions.

But this method is generally much less convenient than the use of permanent colour standards, and is not the subject of the present paper.

Returning to the method based on coloured glass standards, it may be interesting to refer to an experiment designed to test whether relative humidities differing by as little as 1 per cent. could be distinguished by this method. Papers cut from the same piece were exposed for two hours in bottles at 20°C. over solutions to give respectively 80, 81, 82, 83 and 85 per cent. R.H. After being mounted, each paper was matched four times against the glass standards, with the following results:—

84.5, 84.5, 84, 84	:	mean 84.25, for 85 per cent.
83, 82.5, 83, 83	:	82.9, for 83 "
81.5, 81, 81, 81.5	:	81.25, for 82 "
80.5, 80.5, 80.5, 80.5	:	80.5, for 81 "
79.5, 79.5, 80, 79.5	:	79.6, for 80 "

It will be noted that the differences were indicated with a reasonably high degree of accuracy, which could be increased by using several papers at each humidity. The same degree of accuracy could be expected in any part of the range, 65–90 per cent. R.H., and perhaps somewhat beyond these limits, but not, for example, at 30 or 40 per cent. R.H., where the blue colours are not so clearly distinct.

Less emphasis is placed on the accuracy of the absolute values in the above results, since one could not rely on achieving this level of precision without personally re-calibrating the standards against pieces of the same paper as is used in the tests.

Summary.

The method of measuring relative humidity by matching the colours of tissue paper impregnated with cobalt thiocyanate has now been made more convenient by the commercial production of impregnated paper and coloured glass standards.

Although the method as used straightforwardly is not at all precise, it is especially useful for the measurement of humidity in small or relatively inaccessible spaces, *e.g.*, cracks in floors, air spaces in stored grain, under bark, or inside small tubes, etc., and for general use when elaborate equipment cannot be employed.

A piece of the paper is exposed to the humidity to be measured, preferably for two hours, then mounted on white opal glass in oil (liquid paraffin) and matched, in a simple comparator, against the coloured glass standards. When matching is done at a temperature other than 20°C., corrections are required as tabulated.

Tables of error are given, showing the range of variation from different causes, with estimates of 95 per cent. confidence limits. To cover all the sources of variation normally affecting the measurements under various conditions, limits up to ± 5 are allowed for relative humidities down to 70 per cent.; these limits increase at lower humidities to a maximum of ± 15 , about 30 per cent. Various ways of avoiding errors are described, and it is shown that if special precautions are taken the method can be used with considerable accuracy, particularly at relative humidities above 65 per cent.

Acknowledgements.

The impregnated paper used in these tests was produced by Mr. A. G. Hill and his staff at the British Drug Houses Ltd., after considerable difficulties in devising a method of producing large quantities.

The coloured glass standards were produced by Tintometer Ltd. of Salisbury at the conclusion of a long series of trials in collaboration with the author, and

were used in their standard Lovibond Comparator and special stand for grading surface colours.

I am indebted to both firms for their sustained efforts to produce the materials for this method in a generally available and convenient form.

For guidance in statistical matters I am indebted to my colleague, Mr. R. W. Howe. A number of colleagues took part in the tests of individual differences in colour matching.

The method was developed primarily to meet the needs of research at the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

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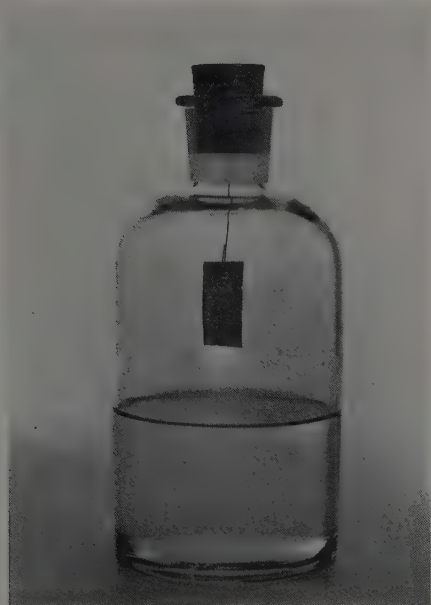


FIG. 1. Cobalt thiocyanate paper hanging on wire from stopper over constant humidity solution.

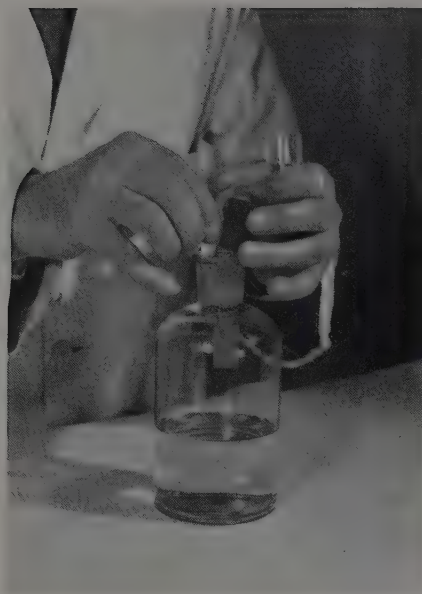


FIG. 2. Paper being pulled downwards from wire with forceps. Tube of oil ready for prompt immersion of paper.



FIG. 3. Oiled paper being doubled over on slip of white opal glass, preparatory to covering with the clear glass. Two fully mounted papers also shown.



FIG. 4. A mounted paper being matched against the glass standards, one disc of which is in the top of the comparator; the second one and an extra disc are on the bench, together with two more mounted papers.

LOUSE CONTROL THROUGH TEXTILE FIBRE SIZE.

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AF.

(PLATES XII-XIV.)

During the last war, but before the advent of DDT, louse control was a problem in situations where the available chemicals were limited to the simplest materials such as naphthalene, sulphur and vinegar. In these circumstances, the possibility of making things uncomfortable for body lice, *Pediculus humanus humanus* L., by wearing clothing made of fibres too thick for them to get their claws around, seemed attractive. The idea lost its attraction when DDT became available, to regain it when lice resistant to DDT were reported (Hurlbut, Altman & Nibley, 1952; Eddy, 1953). Buxton (1947) cited Wigglesworth (1941) and Hase (1915) in showing the influence on lice of variation in fibre-thickness, but, at least within the size range of natural fibres, considered this of small importance. The recent appearance of many synthetic fibres (Larose, 1945), available in various diameters, encouraged this preliminary investigation of the possibilities of such an approach.

Size of Claw and Diameter of Host Hair.

If this idea were to be of value, then correlation between the size of the claws in the various species of lice and the diameter of the body hairs of their mammalian hosts would be expected. Hopkins (1943) found a correlation between coat texture of antelope host and genus of Trichodectid (Mallophaga) parasites, and discussed (1949) the influence of coat on host selection in both biting and sucking lice. A cursory examination of the claws of twelve species of Siphunculata available here indicated that their size was closely related to thickness of host hair; but the measurement of this correlation over a wide range of hair-diameters proved impossible, on account of the difference between the louse sexes and the different pairs of legs, the difficulty in selecting a suitable dimension of the claw for measurement, and the variation in diameter of host hair with the type of hair and the part of the body. The nature of the relationship is shown in Plate XII; hair-diameters in this are taken from Hausmann (1920), Toldt (1935) and Lochte (1938). In this connection it should be mentioned that most mammalian hair is slightly elliptical in section; where this is well marked, diameters shown represent the mean of the two axes of the ellipse. Arising out of these observations it was found that the claws of the front legs of males of *P. humanus* can only encompass the hind femora of the female at the notch behind the femoral spur (Nuttall, 1917) where they are a beautiful fit (fig. 1, d). This notch, difficult to see in a whole mount from dorsal aspect, has only rarely been figured.

It seems that the ability of the louse claw to become adapted to different diameters of host hair is limited at both ends of the range of these. Both at the extreme upper and lower limits of diameter of mammalian hair, the Sirenia and the Chiroptera (hair-diameter of dugong, 1177μ , of *Mormops*, 6.8μ ; Hausmann, 1920), lice have been unable to succeed (Hopkins, 1949). Near both the upper and the lower limits, grip has become the function of the head; the elephant louse, *Haematomyzus elephantis* Piaget (Mallophaga), clearly employs the proboscis for this purpose. This structure is very well developed and strangely

reminiscent of the hypostome of a tick. Many of the Trichodectid Mallophaga on small rodents hold a host hair in a groove of appropriate width in the head with the hypognathous mandibles. The sparseness of the hair in the Sirenia and the elephants has undoubtedly been important too.

The Culture of Lice.

Lice, *Pediculus humanus humanus*, were reared on $1\frac{1}{2}$ -in. squares of black wool baratheia stored in transparent plastic boxes $1\frac{1}{2} \times 1\frac{1}{2} \times \frac{3}{4}$ in. In the top and bottom of the boxes were $1\frac{3}{8}$ -in. holes covered with silk chiffon cemented to the outside of the box. The boxes were worn by the author and volunteers during the day, held against various parts of the body by elastic bandages. At night they were left at room temperature. First-instar nymphs fed quite well through the chiffon, but in the early stages, when a population was being built up, it was found preferable to feed them directly on the skin. It was easier to keep track of the colourless unfed nymphs if the skin were first darkened by rubbing on a mixture of cosmetic lampblack and unguentum lyophilium (Anon., 1949).

About ten pairs of adult lice were maintained in each box. When many eggs had been laid, the lice were transferred to new baratheia squares, and the squares carrying eggs were put in a desiccator. This contained a saturated solution of sodium chloride to keep the relative humidity at 76 per cent., and was held at a temperature of 85°F. Lice which were in use for experiments were fed directly

TABLE I.

The characteristics of the fabrics on which the grip and grab of lice were measured.
(Diameters in microns.)

Fabric	Fibre	Warp			Weft		
		Fibre diameter	Approx. thread diameter	No. of threads per cm.	Fibre diameter	Approx. thread diameter	No. of threads per cm.
1. Drill (ironed)	Cotton	8-19	270	37	8-19	305	23
2. Drill (worn)	Cotton	8-19	270	37	8-19	305	23
3. Bolting cloth	Silk	9-15	75	50 (double threads)	9-15	75	50 (double threads)
4. Baratheia	Wool	13-32	210	28	13-32	450	40
5. Tulle	Nylon	43-45	—	30	43-45	—	30
6. Saran 60 × 60	Saran	127-135	—	23	127-135	—	23
7. „ 120 × 56	„	214-225	—	46	236-255* × 127-135	—	19
8. „ 52 × 52	„	214-225	—	20	214-225	—	20
9. „ 90 × 40	„	255-277	—	35	255-277	—	16
10. „ Cellular	„	378-418	—	10	378-418	—	10
11. „ 8 × 8	„	504-516	—	3	504-516	—	3

* Fibres flattened.

on the skin on the inside of the forearm for 15 minutes twice daily, beginning at 0900 hr. and 1700 hr.

Nymphs, especially the earlier stages, showed a reluctance to feed and a tendency to wander when placed directly on the skin of a person on whom they had not previously fed. For example, 12 to 20 individuals out of a batch of 50 would move 3 to 4 in. from the cloth square on which they were placed on the skin. This tendency was almost completely lost after the first feed on the fresh host.

The Fabrics tested.

In selecting fabrics for test, the objective was to include a wide range of fibre-diameter, extending above that which the claws appeared able to grasp, and to include both fibres conventionally used in clothing and new synthetic materials. Beyond this, little selection was possible; colour and weave were largely dictated by availability.

The 11 fabrics used are listed, with their principal characteristics, in Table I, and four of them are illustrated in Plate XIII. The fabrics are arranged in order of fibre-diameter and are numbered serially. These numbers are used in the other Tables and in fig. 1.

The Measurement of Grip.

The force with which lice could cling to the fabrics was measured with the equipment shown in Plate XIV, fig. 1. A fine silk thread was tied around the louse in between the last two pairs of legs using a slip knot. This was done just after a meal; no anaesthesia was then needed, and there was no undue constriction of the body at subsequent meals. When the knot had been adjusted to the required tightness it was sealed with a droplet of wax applied with an electrically heated nichrome wire loop.

The louse was placed in the centre of a square of the fabric which was clipped to a plastic stage, with a hole in the centre, on the rack and pinion movement from a binocular microscope. A loop on the other end of the thread was placed on the hook of a torsion balance reading to 1 g. by 2-mg. divisions. The rack and pinion movement was then used to bring the index of the torsion balance to zero, and to maintain its position there by compensating for the slight stretch in the thread and in the fabric as tension was applied. Tension was applied to the thread and increased at a uniform rate by engaging the clutch of a kymograph motor which was arranged so as to advance the arm of the torsion balance. Various rates of tension increase were tried and 28.8 dynes per second was found to give the most consistent results; all the data used were obtained at this rate. Electrical contacts were arranged so that when the louse released its hold, the arm of the torsion balance completed a circuit through a relay which switched off the kymograph motor. There was a small overrun, which was constant at any given rate. The tension in the thread at the time that the louse released its hold was obtained by subtracting this correction from the reading on the dial of the torsion balance.

At each series of tests, one louse specimen was used to obtain five readings on each fabric; the fabrics were taken in random order. No tests were made with nymphs; twelve females and six males of various ages were used; no louse was used for more than two sets of readings on any one day. A total of 150 readings was obtained on each fabric, 100 with females and 50 with males. The results are summarised in Table II and fig. 1.

A binocular microscope was arranged so that the lice could be closely observed while clinging to the fabric. It was found that if the fibres were too large to be encompassed by the claws the insects would sometimes obtain their hold by

TABLE II.

The force, in dynes, with which lice can grip various fabrics, and the percentage of lice grabbing the surface of fabrics inclined to the horizontal at different angles.

Fabric	Male grip	Female grip	Percentage of lice holding at inclinations of :							Percentage holding at all angles collectively		Overall percentage holding		
			10°	20°	30°	40°	50°	60°	70°	80°	♂♂		♀♀ Nymphs	
1. Drill (ironed)	109 ± 25.3*	166 ± 17.4	94	71	47	20	16	0	0	24	32	35	31	
2. Drill (worn)	186 ± 26.9	239 ± 25.7	97	72	40	23	30	17	10	7	25	31	54	35
3. Bolting cloth	315 ± 37.0	389 ± 25.7	100	83	40	33	16	10	7	3	34	36	40	37
4. Barathea	401 ± 22.1	376 ± 32.7	100	93	83	50	40	33	40	23	54	60	58	53
5. Tulle	355 ± 28.8	454 ± 34.0	100	83	53	30	11	5	5	0	31	31	39	34
6. Saran 60 × 60	192 ± 31.3	283 ± 14.7	97	73	33	7	10	7	3	0	20	34	33	29
7. " 120 × 56	185 ± 38.7	158 ± 14.4	80	37	20	7	3	0	0	0	13	16	26	18
8. " 52 × 52	142 ± 17.7	66 ± 5.38	90	57	30	10	3	0	0	0	19	25	28	24
9. " 90 × 40	39 ± 9.18	65 ± 8.50	97	57	47	17	3	0	0	0	18	31	34	23
10. " Cellular	43 ± 5.38	65 ± 5.80												
11. " 8 × 8	27 ± 2.53	55 ± 4.25												
Average, all materials	252	181									26	33	39	

* Mean ± standard error of the mean.

clasping the fibre with one or more pairs of legs if the weave were open enough to permit this. A note was made of the readings obtained when this grip was used; they were always much lower than those obtained on other fabrics when a claw grip was achieved.

A few tests were run with the louse in darkness, the stage being placed inside a box with the thread passing through a small hole in the top. Other

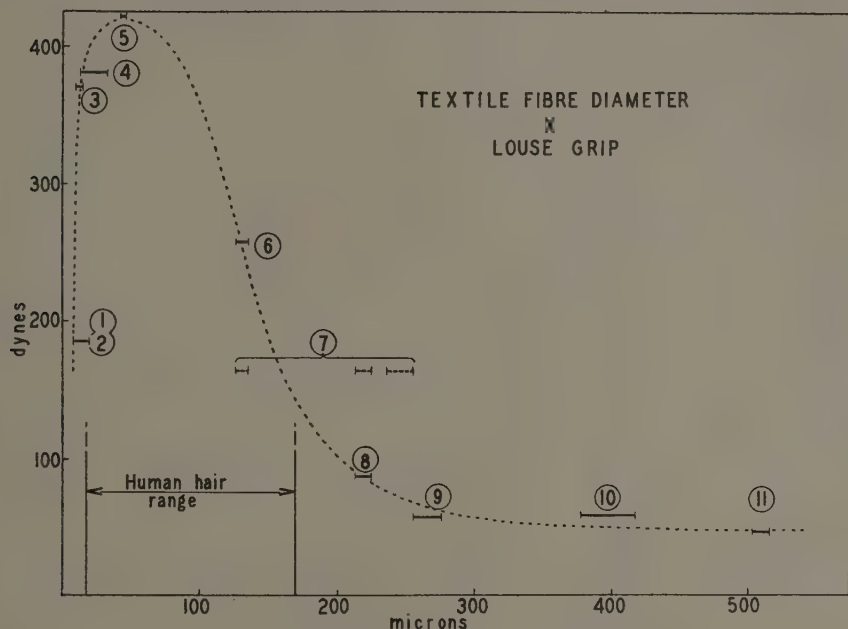


Fig. 1.—Textile fibre-diameter \times louse grip.

tests were run with recently fed lice, with lice starved for 24 hours, with recently emerged lice (12 hr.), and with lice of two weeks' adult age. No differences due to these factors were discernible.

In a few instances, both on baratheia and on nylon tulle, female lice retained their hold up to the maximum capacity of the instrument, 981 dynes less the weight of the louse and harness. The average weight of female lice used was 2.27 mg., that of males 1.34 mg. The average weight of the harness was 0.25 mg. The average grip when a leg hold was used was 41 dynes.

The weft fibres of the 120 \times 56 saran were ribbon-like, flattened in the plane of the fabric; individual readings obtained on this fabric were always higher when the claws gripped the edges of these fibres where the radius of curvature was less, lower when the thicker warp fibres were held.

When placed on the large-fibred fabrics, lice would grapple continuously for a hold sometimes for nearly a minute. Similar behaviour was shown when they were placed on smooth surfaces of glass or plastic. The activity was reminiscent of DDT jitters.

The Measurement of Grab.

The ability of lice to grab hold of a fabric surface, in relation to which they were in motion, was measured with the equipment shown in Plate XIV, fig. 2.

Lice were pushed over the curved polished edge of a lucite platform in such a manner that they fell freely for a distance of 5 cm. before landing on the stretched surface of the fabric under test. The fabric squares were clamped on a lower platform so that they could be rotated about a horizontal axis parallel to one edge and 5 cm. from it. This axis was vertically below the point at which the lice were pushed over the edge of the upper platform. The angle of inclination of the fabric platform was measured by means of a half-circle protractor across which a plumb line was suspended. In this way, whatever the inclination of the fabric, lice fell for a distance of five centimetres before contacting it, when, if they failed to hold at once, they slid, rolled, or bounced over it for a further 5 cm. before leaving its lower edge and falling freely into a dish placed below.

The 8 × 8 and the cellular saran fabrics were not used in these tests because of their open mesh. Adult males, adult females, and nymphs were tested separately on all of the other materials at every ten degrees of inclination from 10 to 80. Ten to 30 specimens of each stage and sex were dropped at each angle on each fabric. The results are given in Table II.

When lice were able to grab the fabric, this was nearly always at the point at which they first contacted it. Some tests with this equipment also were conducted in the dark, but no differences were discernible.

Oviposition.

Since fibres must be grasped by the gonopods for oviposition, fibre-diameter might be expected to influence this function too. During the studies on grip and grab, lice left in contact with the different fabrics overnight laid eggs on all of them except those made of saran, which were all of large-diameter fibres. In a preliminary test in which six gravid females were kept on 90 × 40 saran and wool barathea for alternate periods of 24 hours for six days, 23 eggs in all were laid on the barathea, none on the saran.

TABLE III.

The number of eggs laid by 20 gravid female lice in 24 hours on various fabrics.

Fabric	Test 1—Single discs		Test 2—Double discs		Mean no. of eggs laid
	Eggs laid	Mortality	Eggs laid	Mortality	
2. Drill (worn) ..	65	0	60	1	62.5
4. Barathea* ..	22	12	68	0	68
6. Saran 60 × 60	52	2	53	2	52.5
7. „ 120 × 56	41	2	36	1	38.5
8. „ 52 × 52	45	2	29	0	37
9. „ 90 × 40	28	3	32	1	30
0. Khaki wool cloth	76	0	79	0	77.5

* Cause of mortality in test 1 unknown; replaced by wool cloth (0) dyed black, in test 2.

By arrangement with W. C. McDuffie at the U.S.D.A. laboratory at Orlando, Florida, the following further tests were made by I. H. Gilbert. Twenty gravid female lice were placed on 1½-in. diameter discs of the fabrics in 50 ml. beakers and held at 82 to 85°F. and 70 per cent. R.H. for 24 hours. In a second test, two discs of the same fabric were used in each beaker. The only choice of

oviposition surface was between glass and the fabric. In each series, standard khaki wool cloth was included for comparison; the fibre-diameter in this ranged from 13 to 34 microns. Results are shown in Table III.

Discussion.

The diameter of human hair, according to Jackson & McMurtry (1912, p. 47) ranges from about 17 to 170 microns. This includes all kinds of hair, body hairs are rarely more than 100 microns thick, and average about 60 microns. The fibres for which the highest grip figures were obtained all had diameters within this range. Fibres over 200 microns in diameter do not appear to be very suitable for the attachment and locomotion of lice. Curves drawn as in fig. 1, but for each sex separately, show that the peak grip force by females is found at 43 microns fibre-diameter, that of males at 25 microns. Grip is apparently reduced on very small fibre-diameters as well as on large ones. This is not due to breakage of the fibres however; lice were never found to have the broken ends of fibres in their claws when pulled off fabrics. It may be supposed that the mechanical advantage of the unguitractor muscles is reduced when the claws have to be closed far enough to grip these fibres. The maximum grip forces recorded (in dynes) are about 440 times the weight of the louse (in mg.). The average grip which can be obtained with the leg hold is less than a tenth of that obtained on suitable fibres with the claws.

The ability of lice to grab hold of fabrics varies with fibre-diameter in a similar manner to grip, but rather less strongly. Weave has more influence here; the lowest figures for grab are those for the most closely woven saran material, 120×56 , despite its relatively small fibre-diameter.

If the number of eggs laid is plotted against fibre-diameter (Table III), a curve of similar shape to that in fig. 1 can be drawn.

Fibres thick enough to present difficulties to lice do not seem likely to produce comfortable clothing; when a shirt made of 120×56 saran was worn next to the skin, the cut ends of fibres at the seams were the main source of discomfort. A more flexible fibre, in a suitable weave, might well prove satisfactory, and it would certainly be difficult for lice to colonise and maintain themselves on it. Possibly very fine fibres would also be an improvement over conventional materials if suitably spun and woven so that the threads did not provide easy claw holds. The value of starching and ironing drill in minimising the likelihood of picking up and carrying lice is clearly shown in Table II. Perhaps the most promising application for fabrics of unusual fibre diameter would be in outer garments for medical staff handling a typhus epidemic. The principle is used in reverse by eskimos who catch lice under the clothing by inserting a tuft of bear fur tied to a stick. After an appropriate interval the louse is withdrawn with the fur. The diameter of polar bear hair is very close to that of human hair.

There appears no doubt that hair-diameter is an important factor in host selection by lice. Perhaps louse-proof breeds of domestic animals can be developed by selecting for thicker or thinner hair, or even, in some instances, hairlessness. Allen & Dicke (1953) have shown that some cattle lice can be controlled by clipping.

Summary.

The possibility of controlling the human body louse, *Pediculus humanus humanus* L., through the development of clothing which is inimical to it on physical rather than chemical grounds was investigated. Claw size in lice was shown to be related to diameter of host hair. The grip force of lice on fabrics made of fibres of various diameters and compositions, some spun and some unspun, and of various weaves largely dictated by availability, was measured with an apparatus of which the essential parts were a torsion balance and a kymograph

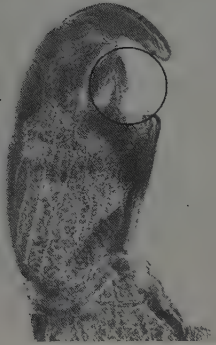
motor. The force was found to be a maximum at fibre-diameters approximating that of human hair. The ability of lice to grab hold of a fabric when dropped on to an inclined surface of it was also measured and found to vary with fibre-diameter in a similar manner to grip, but less strongly. The number of eggs laid on fabrics when no choice was offered was also found to vary with fibre-diameter in a similar manner. While this physical method of louse control is not promising for immediate practical application, it has possibilities which may be enhanced by developments in textile technology.

Acknowledgements.

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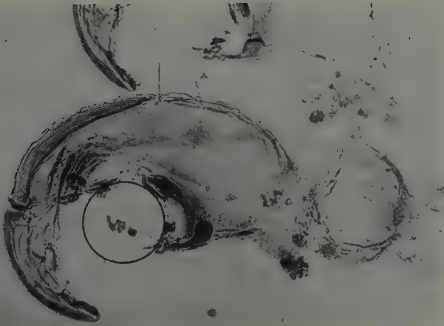
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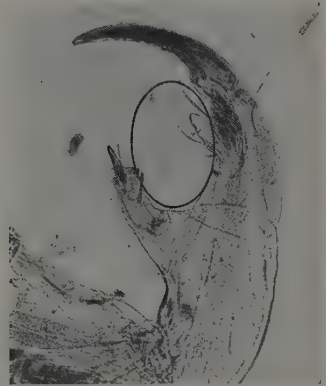
(a)



(b)



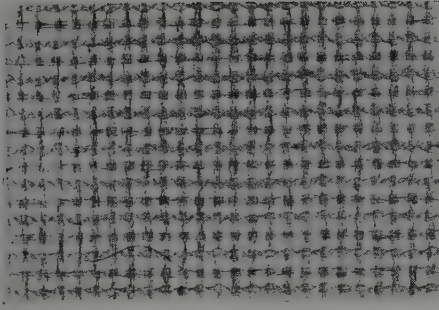
(c)



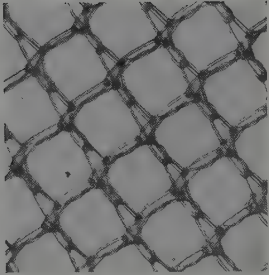
(d)

The relationship between size of louse claw and diameter of host hair. The inscribed circles represent the average hair-diameter of the host-animal. In (c) this is the diameter of pubic, axillary, eyelash and beard hairs. The ellipse in (d) represents the size of the hind femur of the female at the notch behind the femoral spur. All are $\times 100$.

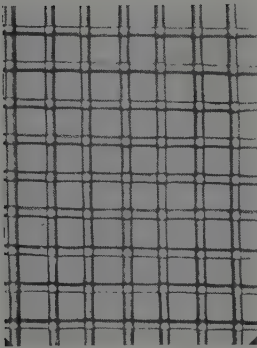
(a) *Haematopinus asini* (L.) ♀, mesothoracic foot; (b) *Haematopinus suis* (L.) ♀, prothoracic foot; (c) *Phthirus pubis* (L.) ♀, metathoracic foot; (d) *Pediculus humanus* (L.) ♂, prothoracic foot.



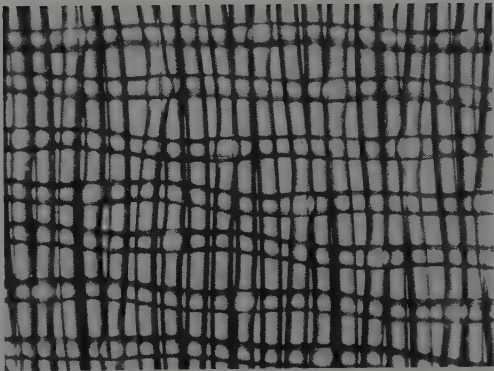
(a)



(b)



(c)



(d)

Four of the fabrics tested ($\times 12\frac{1}{2}$).

- | | |
|---------------------------|---------------------------|
| (a) Silk bolting cloth; | (b) nylon tulle; |
| (c) 60 \times 60 saran; | (d) 90 \times 40 saran. |

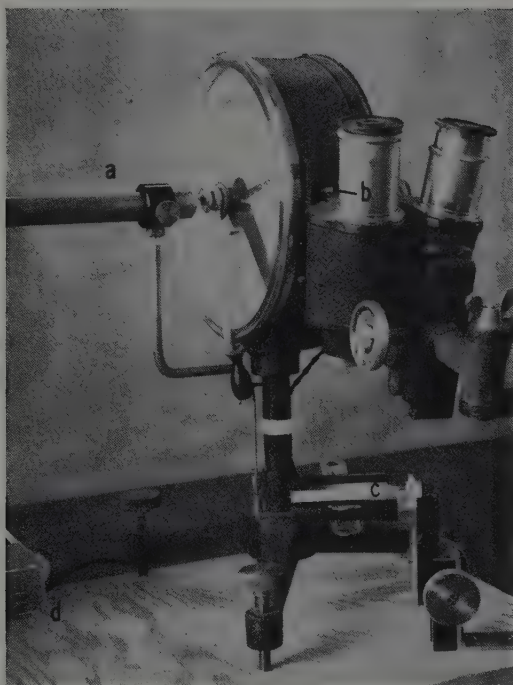


FIG. 1. Equipment used for the measurement of grip. (a) Kymograph shaft with arm operating lever of torsion balance; (b) torsion balance hook from which lice were suspended; (c) fabric sample clipped to stage on rack and pinion; (d) relay to switch off kymograph.

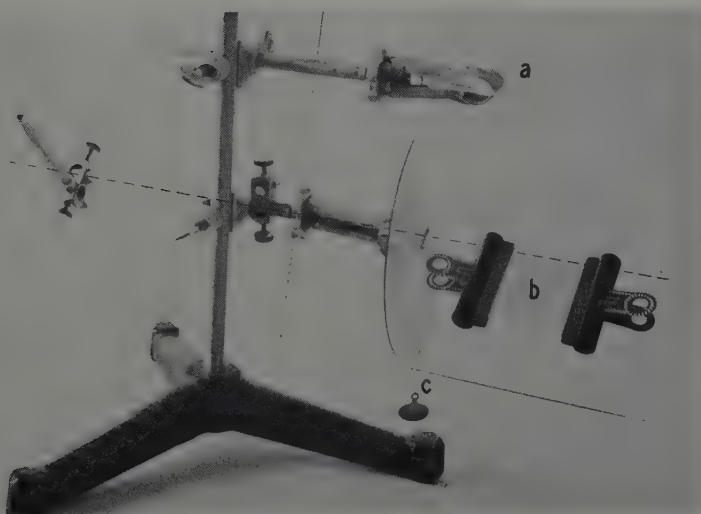


FIG. 2. Equipment used for the measurement of grab. (a) Lucite platform from which lice were pushed; (b) fabric sample clipped to stage rotating about axis shown by broken line; (c) plumb line on half-circle protractor.

THE REACTIONS OF THE DESERT LOCUST, *SCHISTOCERCA*
GREGARIA (FORSK.), (ORTHOPTERA, ACRIDIDAE) TO
 PHYSICAL FACTORS, WITH SPECIAL REFERENCE
 TO RELATIVE HUMIDITY.

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In recent years much work has been done on the behaviour of animals in relation to light, temperature and humidity. Many authors have recently considered moisture as a factor affecting locust movements (Volkonsky, 1939; Bodenheimer, 1944; Kennedy, 1939, 1945) and avoidance of moist air has been found in *Locusta migratoria migratorioides* (R. & F.) by Kennedy (1937). The effects of temperature on the Desert Locust, *Schistocerca gregaria* (Forsk.), have been studied by Bodenheimer & others (1929), Fraenkel (1929), Hussein (1937), Kennedy (1939, 1945, 1951) and Volkonsky (1939), who have found positive reactions to temperature. Fraenkel (1929, 1930) Karandikar (1933), Hussein (1937), Kennedy (1939) and Volkonsky (1939) have studied the effects of light on the activity of *S. gregaria* and have described both photokinetic and phototactic behaviour.

From the above accounts and from numerous field observations it appears that physical factors exercise a great influence on the activity of the Desert Locust, and the present experiments were designed to study the simultaneous influence of two or three factors on the movements of the first- and of the fourth-instar hoppers of *S. gregaria* of phase *gregaria*.

Material and Apparatus.

The insects.

Newly hatched first-instar and newly moulted fourth-instar hoppers of *S. gregaria* were obtained regularly from the Anti-Locust Research Centre, London, and were kept in the insect cages of a preconditioning apparatus (described by Dempster, 1953) at 28°C. and 77 per cent. R.H. for three days. They were fed twice daily, morning and evening, on slightly dried grass.

The alternative chamber.

An alternative chamber similar to that described by Gunn & Kennedy (1936) was used. The glass dish was 5.5 cm. high and 30 cm. in diameter. Small petri dishes were arranged symmetrically, so that they covered the whole glass floor and were contiguous along the circumference and along the diameter of the chamber. The desired humidities were maintained by means of measured quantities of potassium hydroxide of suitable concentrations (Solomon, 1951). A false floor of perforated zinc sheet was marked into a number of regions by red lines and was placed over the petri dishes. The edges of the false floor were sealed on to the walls of the chamber with transparent adhesive tape. A glass plate with a central hole covered with a slide, to admit the insects, was placed over the glass dish and made airtight by vaseline. Three such chambers were set up in the evening and left in the constant temperature room for establishment of the humidity gradients. The apparatus was thus designed to give an enclosed space with gradients of humidity. The extreme values of these gradients were measured by Gregory's hygrometer and also checked by the cobalt-thiocyanate-paper method (Solomon, 1945).

Conditions and Methods.

The alternative chamber was placed in a wooden box, about 40 cm. square and 32 cm. high, having two side walls and a roof, the latter with a circular opening, 30 cm. in diameter, which was covered by a piece of milky plastic sheet and a layer of brown tracing paper. The open back of the box was placed against the

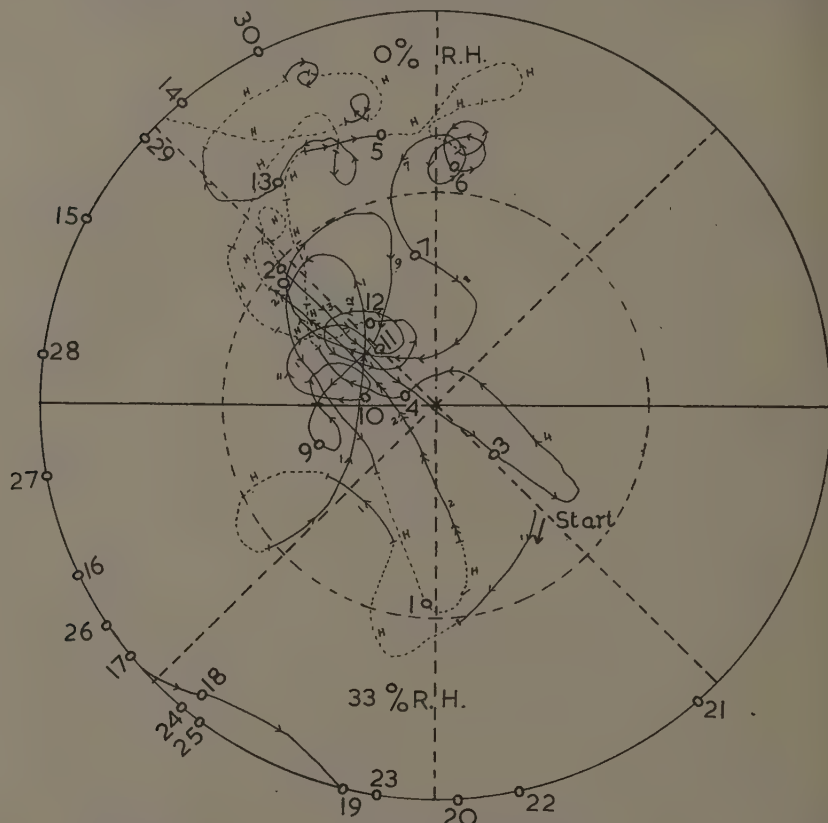


Fig. 1.—Record of the behaviour of one first-instar hopper of *Schistocerca* during 30 minutes in an alternative humidity gradient of 0–33 per cent. R.H. at 30°C. and with a light intensity of 0.7 log foot-lamberts. The numbers associated with small circles show the position of the insect at the end of the corresponding minute. The letter H shows where the insect hopped. The behaviour in this example is summarised as follows:—

R.H. (%)	Totals				Average speed of walking (in./sec.)
	Time spent (sec.)	Time active (sec.)	No. of hops	No. of turnings	
0	874	512	14	10.5	0.154
33	926	554	4	2	0.130

wall of the room while the surface of the table formed the floor of the box. The front of the box was open to allow the recording of observations. A circular glass vessel, 30.5 cm. in diameter and 12.5 cm. high, was filled with cold water and placed over the circular opening of the box. This was arranged to allow the diffused light rays to fall on the false floor of the alternative chamber and also to eliminate radiant heat. For the low light intensity (0.7 log foot-lamberts) one 40 watt tungsten-filament pearl bulb was hung 38 cm. above the glass vessel. This was the only light in the otherwise completely dark room. To obtain the higher light intensity (2.1 L.F.L.) three similar bulbs, each of 150 watt, were used and the brown tracing paper was removed from the top of the box. The intensity of light on the false floor was measured with an S.E.I. Ilford photometer. This arrangement was used to study the reactions of the hoppers to two physical factors, light and humidity, while the third factor, temperature, was controlled at 20° or at 30° C. in a constant temperature room.

Two main series of experiments were conducted:—

(1) Single humidity experiments, with uniform humidity in the chamber. In these experiments, the rates of hopping and turning and the speed of walking, at different single humidities, were compared.

(2) Alternative humidity experiments, where the hoppers were provided with a gradient of humidity in the same chamber, the two sides of which are referred to as the "wet" and the "dry" halves.

To eliminate the possibility of gregarious behaviour, it was essential to use one hopper at a time. Ten experiments, each of 30 minutes duration, were done on hoppers previously conditioned at 28°C. and 77 per cent. R.H. for three days. Each insect was released through the central hole of the glass plate which was then covered by the slide. Half an hour was allowed to re-establish the humidity equilibrium. The following observations were then recorded throughout each minute:— (i) time spent "inactive" (*i.e.*, complete rest, no movement at all), (ii) time spent "active" with progress (*i.e.*, walking on the false floor, ascending and descending the sides of the chamber and glass plate), (iii) time spent "active" without progress (*i.e.*, movement of legs or body segments, cleaning, etc.), (iv) the number of hops made per minute. The hopper's walking movements on the false floor for each minute were drawn on graph paper. The recorded behaviour of one such hopper is shown in fig. 1. The distance walked was measured with an opisometer and divided by the number of seconds spent in walking to obtain (v) the speed, in inches per second. Ascending and descending movements on the sides of the chamber were marked on the periphery of the false floor. From the same graph, the total number of "turnings" (vi) was also counted in each experiment. A turn through 180° was counted as half a turn, while 360° was counted as one complete turn. Although this must be considered to be an imperfect method of recording klinokinesis, it gave a sufficient indication of the differences in behaviour under different environmental conditions. Possible factors external to the chamber were eliminated by rotating the chamber through 180° after 15 minutes' observation.

In the alternative R.H. experiments, special care was taken to record the activities of the hopper and of the time spent in each half of the chamber. The total time spent in the "wet" and "dry" halves was thus recorded. As an estimate of the intensity of reaction, the "excess percentage of reaction" $\frac{(W-D)}{W+D} \cdot 100$, was used (Gunn & Cosway, 1938). In this expression W and D are the times spent by the hopper in the wet and dry halves, respectively. The theoretical value for no reaction is 0.0 per cent. Intensities of reaction with positive signs denote a preference for high R.H. and *vice versa*.

The ratio between time spent "inactive" and "active" was obtained by

dividing the total time spent "inactive" by the total time spent active. The percentages for total time spent "active" and "inactive" were expressed in two ways: the first way was to express the time active in "dry" as percentage of total time spent in "dry" and similarly for "wet". The second way was expressed as the time active in "dry" as percentage of the total time of the 10 experiments (*i.e.*, of 18,000 seconds). Hops and turnings were expressed as total per unit of time (*i.e.*, of 1,800 seconds in each experiment) and also per unit of time that the hopper was active.

Results.

A. Reactions of first-instar fed hoppers to physical factors.

(1) *Single humidities.*—The reactions of first-instar fed hoppers (3 days old, preconditioned at 28°C. and 77 per cent. R.H.) were studied in single humidities of 0, 33, 66 and 100 per cent., at temperatures of 20 and 30°C. and in light intensities of 0.7 and 2.1 L.F.L. (= log foot-lamberts). The results of ten replicates are summarised in Table I.

Examination of these unanalysed data immediately suggests that both hopping and turning movements of hoppers are affected by humidity, and it is apparent that the hoppers are less active at 66 per cent. R.H. than in the lower humidities of 0 and 33 per cent., and that they become greatly agitated in

TABLE II.

Result of analysis of variance on the reactions of first-instar fed hoppers in single R.H. experiments.

Factors	Time spent active	Number of hops	Hops per unit of time active	Number of turnings	Turnings per unit of time active	Average speed of walking
Temperature	P>0.05	P<0.001*	P<0.001*	P<0.001*	P<0.001*	P<0.001*
Light	P<0.001*	P>0.2	P<0.05*	P>0.2	P>0.2	P<0.001*
Humidity	P>0.05	P<0.001*	P<0.001*	P<0.001*	P<0.001*	P<0.001*
Temperature and light interaction	P=0.2	P>0.2	P>0.2	P<0.01*	P<0.01*	P=0.05*
Temperature and humidity interaction	P=0.2	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2
Light and humidity interaction	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2
Temperature, light and humidity interaction	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2

* Significant at P = 0.05 or less.

saturated air at 100 per cent. R.H. Examination of this Table also indicates that the speed of walking increases considerably and consistently as the humidity rises, both in conditions of low and of high temperature and light intensity.

However, since the hoppers were subjected to three measured physical factors simultaneously, these results were sorted out statistically by an analysis of

variance (see Table II), from which it was possible to evaluate not only their direct effects, but also those of their interactions.

If one considers the different behaviour patterns one at a time, it may be said that the length of period of general activity in these experiments was affected directly by temperature, light and humidity independently of each other, but most of all by the increase in light intensity.

Hopping was affected by temperature and by humidity, whether one considered the total duration of the experiments, or only the period of time in which the hoppers were active. It may also be considered that the raising of light intensity affected the number of hops, although not so greatly as the other two physical factors, and this result only borders on significance (see Table II, column 4) and only when the hops are considered in the period of time when the locusts were actually moving.

The number of turnings was also affected by temperature and by humidity (Table II, columns 5 & 6) but not by light. However, the effect of the interaction of light and temperature on turnings was significant.

The average speed of walking increased with the progressive rise in humidity, temperature and light. It is interesting to see that the number of turnings was also significantly affected by the interaction of light and temperature and suggestive of some integration of the effects of these physical factors in the nervous system of the insect.

Summarising the results at single humidities, it may be said that the increase in temperature raises the general level of activity, *i.e.*, the insects hop more and walk more rapidly at 30° than at 20°C. Similarly, increased illumination affects the number of hops, the rate of walking and the number of turnings. Humidity has a profound effect on activity, the speed of walking rising directly with this physical factor, while the number of hops and turnings, *i.e.*, the general agitation of the hoppers, decreases at 66 per cent. and then again rises to a peak at 100 per cent. R.H.

(2) *Alternative humidities.*—Three pairs of alternative humidities, of 0 and 33, 33 and 66 and 66 and 100 per cent., were used at 20 and at 30°C., and at light intensities of 0.7 and 2.1 L.F.L. at each temperature. Ten replicates, each of 30 minutes' duration, were done at each pair of relative humidities, at both temperatures and both light intensities. The results are summarised in Table III. The intensities of reaction were also analysed statistically by an analysis of variance and are given in Table IV.

TABLE IV.

Summary of analysis of variance on the intensity of reaction of first-instar hoppers in the alternative R.H. experiments.

Factors	Probability	
Temperature	>0.2	
Light	>0.2	
Humidity	<0.001	Very highly significant
Temperature and light interaction	>0.2	
Temperature and humidity interaction ..	>0.2	
Light and humidity interaction	>0.2	
Temperature, light and humidity interaction	>0.2	

In these experiments the hoppers spent slightly more time in the wet half of the apparatus up to 66 per cent.; but in the alternative of 66 and 100 per cent. they exhibited a reversal of action. This humidity effect is significant and is not influenced by light and temperature (see Table IV). Hopping and turning activities are greater in the dry halves up to 66 per cent. R.H., but with alternatives of 66 and 100 per cent. R.H. they become greater in the wet half. The minimum of hopping and turning activity at 66 per cent. R.H. tends to confine the hoppers to this humidity zone. The speed of walking, however, increases with the rise of humidity.

The observations on the above experiments at two different light intensities show a clear increase in the activity of the hoppers, in both halves, at high light intensity. The speed of walking also increases with the rise in light intensity. This shows that high light stimulates activity, whereas the speed of walking of hoppers is influenced both by the increase of light and of humidity.

The number of hops in high light-intensity experiments with 0 and 33 per cent. and 66 and 100 per cent. R.H. at 20°C. is high as compared to that in the experiment with 33 and 66 per cent. gradient at 20°C., while this activity is at a higher level at 2.1 than at 0.7 L.F.L. at 30°C. The difference in the number of hops at high and at low light intensities would lead one to believe that increased illumination affects this form of activity, but in fact the hops are influenced by humidity and not by light. This can be most clearly seen from the negligible difference in their number in the wet halves of the apparatus in the alternatives of 33 and 66 per cent. R.H. in the two different light intensities at 20°C., while the increase in this activity in the same gradient (33 to 66 per cent. R.H.) at the high light intensity at 30°C. may be attributed to the effect of temperature and not of light.

The number of turnings is slightly greater in the higher light at 20°C., while it is nearly identical in the two light intensities at 30°C. One may conclude that the increase in light intensity has the least effect on the turning movements, or klinokinesis of these hoppers. This activity is controlled by humidity and not by light.

At 30°C., the hoppers still tend to remain in the wet half of the apparatus in gradients of 0 to 33 and 33 to 66 per cent. R.H. However, at low light intensity they spend less time in the wet half of the apparatus than in the corresponding experiments at 20°C., and more time at high light intensity than in the corresponding experiments at 20°C. When the alternative humidities are 66 and 100 per cent., the hoppers behave similarly in the two temperatures when the light intensity is low, *i.e.*, there is no marked effect of temperature on the excess percentage of the reaction. At a higher light intensity, the hoppers spend less time in saturated air at 30° than they do at 20°C.

At low light, the effect of temperature on the "time spent active" is clear, whereas the duration of activity is almost identical at the two temperatures when the intensity of light is increased. Thus, the effect of temperature on the duration of activity is not so marked as that of light. However, the effect of the increase of temperature from 20 to 30°C. can be very well detected by the increased speed of walking and by the increased number of hops and turnings.

(3) *Zone of minimum activity.*—As has been obvious from the foregoing experiments carried out in single humidities and in gradients of humidity, there was a marked decrease in some behaviour patterns, namely in the number of hops and turnings, at 66 per cent. R.H. Below this relative humidity, and even more strikingly above it, the hoppers became more agitated. It was considered that this humidity was near to the "zone of minimum activity", *i.e.*, the zone of humidity in which the hoppers appeared to be least agitated, and more detailed experiments with alternative humidities were designed in the hope of obtaining a more precise definition of this zone. The alternatives presented to the hoppers

TABLE V.

Result of ten replicates, one fourth-instar fed hopper in each experiment, at each R.H., temperature and light intensity.
(Single R.H. Experiments.)

	20°C.						30°C.					
	0.7 L.F.L.			2.1 L.F.L.			0.7 L.F.L.			2.1 L.F.L.		
	0	R.H. (%) 33 66 100		0	R.H. (%) 33 66 100		0	R.H. (%) 33 66 100		0	R.H. (%) 33 66 100	
Percentage of time active	55.9	59.1	61.4	63.4	61.5	63.1	60.8	62.4	61.7	64.1	65.9	64.6
Ratio of time inactive/active ..	0.78	0.69	0.62	0.57	0.62	0.58	0.64	0.60	0.62	0.56	0.51	0.54
Total number of hops ..	428	28	9	23	1	10	3	4	4	14	29	1
Hops per unit of time active ..	0.23	1.4	0.6	1.5	0.07	0.54	0.20	0.25	0.26	0.85	2.1	0.043
Total number of turnings	50.5	62	35	73.5	56	77.5	84	83	49.5	106	73.5	71
Turnings per unit of time active ..	2.8	3.5	2.1	4.9	3.5	4.9	5.1	5.4	3.8	6.9	4.8	4.5
Average speed of walking (in. per sec.) ..	0.399	0.464	0.529	0.642	0.424	0.525	0.464	0.511	0.545	0.673	0.542	0.610
												0.670

were 50 and 60, 60 and 70, 70 and 75 per cent. R.H., at 20°C. and at light intensity of 2.1 L.F.L. Three-day-old, fed, first-instar hoppers preconditioned at 28°C. and at 77 per cent. R.H. were again used. In this set of experiments the zone of minimum activity was found to be between 65 and 70 per cent. R.H., and the hoppers remained longer and became more quiescent in the half of the apparatus at 70 per cent. Above 70 per cent. R.H., the hoppers were agitated by the higher humidity and their speed of walking and the number of hops and turns increased. They were able to discriminate between the wet and the dry halves of the apparatus even with a 5 per cent. difference between the humidity extremes.

B. Reactions of fourth-instar fed hoppers to physical factors.

(1) *Single humidities.*—Fourth-instar fed hoppers of *S. gregaria* (3 days old, preconditioned at 28°C. and 77 per cent. R.H.) were used in the same experimental conditions as described for the first-instar hoppers. The results are summarised in Table V while the results of analysis of variance are given in Table VI.

TABLE VI.

Result of analysis of variance on the reactions of fourth-instar fed hoppers in single R.H. experiments.

Factors	Time active	Number of hops	Hops per unit of time active	Number of turnings	Turnings per unit of time active	Average speed of walking
Temperature	P<0.05*	P>0.2	P>0.2	P<0.01*	P=0.001*	P<0.001*
Light	P<0.001*	P>0.2	P>0.2	P>0.2	P>0.2	P<0.001*
Humidity	P>0.05	P<0.05*	P<0.05*	P<0.01*	P=0.001*	P<0.001*
Temperature and humidity interaction	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2	P<0.001*
Temperature and light interaction	P>0.2	P<0.05*	P<0.01*	P>0.2	P>0.2	P>0.2
Light and humidity interaction	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2	P<0.001*
Temperature, light and humidity interaction	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2	P>0.2

* Significant at P = 0.05 or less.

From these Tables one may conclude that the activity (time active) is affected by increase in temperature and light. Speed of walking is influenced by the rise in humidity, temperature and light; while hops are influenced by rise in humidity. Turnings are affected by increase in temperature and humidity. But the hopping and turning activities are again the least at 66 per cent. R.H.

The results of statistical analysis are similar to those of first-instar hoppers except for the following differences:—

- (i) "Time active" is significantly affected by temperature ($P > 0.05$).
- (ii) Number of hops and hops per unit time active are not significantly affected by temperature ($P > 0.2$).
- (iii) Number of turnings and turnings per unit time active are not significantly affected by the interaction of light and temperature ($P > 0.2$).
- (iv) The speed of walking is not significantly affected by the interaction of temperature and light ($P > 0.2$), but it is significantly affected by the interactions of temperature and humidity and also by light and humidity ($P > 0.001$).

(2) *Alternative humidities*.—Reactions of fourth-instar hoppers were studied in the same experimental conditions as were described for the first instar. The summarised results are given in Table VII. The excess percentage of reactions were subjected to an analysis of variance, and the results were similar to those obtained from the first-instar hoppers.

The hoppers remained longer in the higher humidity up to 66 per cent., but again, beyond 66 per cent. R.H., they exhibited a reversal of the reaction and spent more time at the lower humidity. As in the first instar, turning and hopping were the least at 66 per cent. R.H., while the speed of walking was always greater in the wet half of the apparatus.

On comparing the results of the excess percentage of reaction (*i.e.*, time spent active in the wet half) at 20° and at 30°C. at low light intensity, one finds an increase in the reaction in the alternative humidities of 0 and 33 and of 66 and 100, and a decrease in the reaction at 33 and 66 per cent. R.H. at the higher temperature. In the higher light intensity this excess percentage of the reaction is identical at 20 and at 30°C. when the alternative relative humidities are 0 and 33, greater at 30° than at 20°C. in alternative humidities of 33 and 66, and less at 30° than at 20°C. when the humidity gradient is between 66 per cent. and saturated air.

On comparing the activity of the fourth-instar hoppers at 20 and at 30°C., one sees that they are slightly more active at the higher temperature and at the lower light intensity in the alternative humidities of 0 and 33 per cent., and 33 and 66 per cent., and are equally active at the two temperatures when the alternative relative humidities are 66 and 100 per cent. In the higher light intensity, the hoppers are more active at higher temperature in the alternative humidities of 0 and 33 per cent., while their activity is identical at the two temperatures in the alternatives of 33 and 66 and 66 and 100 per cent. R.H.

Speed is affected by increase in temperature, light and humidity.

The number of hops is less at low light intensity in the increased temperature, while in high light it is identical in the gradients of 0 and 33 per cent. R.H., and 33 and 66 per cent. R.H., and is at a higher level in the gradient of 66 and 100 per cent. R.H. at 30°C. than at 20°C.

The number of turnings in the gradient of 0 and 33 per cent. R.H. at low light is greater at 20 than at 30°C., while it is greater in the gradients of 33 and 66 per cent. R.H., and 66 and 100 per cent. R.H. at 30°C. Thus in high light and in the increased temperature this activity is at a slightly higher level.

(3) *Zone of minimum activity*.—As in the first-instar hoppers, it was seen that the numbers of hops and turnings were the least at 66 per cent. R.H. both in experiments with constant humidities and in experiments where alternative humidities were presented. Experiments were done with alternative percentages of R.H. of 55 and 60, and of 60 and 65 at 20°C. at light intensity of 2.1 L.F.L. and it was found that the hoppers spent more time at 60 per cent. R.H., but above this humidity they exhibited a reversal of reaction and returned to this zone. It would thus seem that the zone of minimum activity of the fourth instar, as in the first instar, is determined by humidity, but whereas in the first

TABLE VII.

Reactions of fourth-instar fed hoppers in the alternative R.H. experiments at 20 and 30°C.
and light intensities of 0.7 and 2.1 log foot-lamberts.

	20°C.												30°C.											
	0.7 L.F.L.						2.1 L.F.L.						0.7 L.F.L.						2.1 L.F.L.					
	R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)		R.H. (%)	
	0	33	66	100	0	33	66	100	0	33	66	100	0	33	66	100	0	33	66	100	0	33	66	100
Percentage of time spent in each half	44.1	55.9	41.1	58.9	59.1	40.9	45.6	54.4	46.4	53.6	58.7	41.3	42.8	57.2	43.2	56.8	61.3	38.7	46.2	53.8	44.4	55.6	53.9	46.1
Percentage of time spent active ..	53.8	55.3	59.2	62.5	68.0	63.8	60.4	62.9	63.5	65.9	65.7	66.8	60.3	60.2	62.4	61.1	63.5	64.8	65.3	65.0	64.2	64.1	62.1	65.2
Ratio of time inactive/active	23.8	29.9	24.3	36.8	37.2	26.0	27.6	34.7	29.5	35.3	38.7	27.6	25.8	36.43	27.0	34.7	39.0	25.1	30.2	35.0	23.5	35.05	33.5	30.0
	0.85	0.80	0.63	0.59	0.59	0.57	0.64	0.61	0.57	0.51	0.52	0.49	0.65	0.66	0.60	0.63	0.57	0.54	0.53	0.53	0.55	0.56	0.61	0.53
Total number of hops ..	5	0	9	7	20	36	0	0	4	2	0	1	2	0	0	1	0	2	3	2	6	2	4	30
Total number of turnings ..	37	38.5	36	26	22.5	36	29	34	36.5	30	38	48	29	33.5	45.5	34	46	57	44.5	44	56	41.5	39	59.5
Average speed of walking (ins. per sec.) ..	0.354	0.467	0.458	0.570	0.524	0.641	0.433	0.519	0.529	0.614	0.617	0.622	0.434	0.559	0.538	0.585	0.571	0.676	0.554	0.553	0.606	0.612	0.617	0.662
Excess percentage of reaction ..	11.7	17.7			-18.2	8.81		7.15		-17.43	14.5	13.6		-22.5	7.6	11.1								-7.8

instar it was around 70 per cent., it was around 60 per cent. R.H. in the older hoppers.

A more complete comparison between the reactions of first- and fourth-instar hoppers may now be drawn. On page 525 are listed some of the differences between their reactions. The main difference is seen to be their reaction to temperature, which in fourth-instar hoppers increases the proportion of time spent active but has no effect on the rate of hopping, while the converse is true for first-instar hoppers. The interactions of light and temperature seen in the first instar (Table II) are replaced in the fourth instar by a different set of interactions (Table VI).

Both the first- and fourth-instar hoppers were activated by the low humidities and by the saturated air. They became more quiescent at relative humidities around 60 and 70 per cent. R.H.

Invariably, the fourth-instar hoppers moved faster than did those of the first instar, but the speed of movement is probably a function of size.

Activity, as measured by the number of hops per unit time (*i.e.*, experimental time of 30 minutes), is always lower in the fourth-instar than in the first-instar hoppers. In other words, progression in the first instar is accompanied by a large number of hops whereas the older hoppers hop less and the distance is covered by marching. This observation is confirmed by field observers (*e.g.*, G. Popov (personal communication); Ellis, 1951) and also by Hussein (1937).

It may be here noted that in both the first- and in the fourth-instar hoppers, the intensity of the reaction was in better accord with the humidity differences when these were expressed as relative humidity than as saturation deficits, and throughout this paper it is the effects of relative humidity which are considered.

C. Effect of starvation on first-instar hoppers.

Newly hatched first-instar hoppers were conditioned at 28°C. and 77 per cent. R.H. for three days. They were fed for two days and starved for 24–32 hours. The reactions of the starved hoppers were studied in the percentage R.H. gradients of 65 and 70, 50 and 60, 60 and 70, 70 and 80 and 80 and 90, and 90 and 100, all at 20°C. and light intensity of 2.1 L.F.L. Time spent active, speed of walking, hopping and turnings were at greater levels up to 70–80 per cent. in the starved hoppers than in the fed ones. But beyond 70 and 80 per cent. R.H. there was a decrease in these levels. There was thus a difference in activity of the fed and starved hoppers. The starved hoppers were on the whole more active and excitable. The same conclusion was reached by Ellis (1951). They avoid relative humidities below 60–70 per cent. (as did the fed ones) and, above 70 per cent., avoid the high ones. However, in contrast to the fed hoppers, they were far less active in the moist air (*i.e.*, at 90–100% R.H.), and this, together with their generally greater excitability, constituted the main difference in the behaviour of the fed and the starved hoppers.

D. Effect of conditioning of first- and fourth-instar hoppers.

Newly hatched first-instar and newly moulted fourth-instar hoppers were fed and conditioned at 28°C. and 77 per cent. R.H. separately for two days. On the third day, four different groups were conditioned at low (30%) and high (95%) R.H. for 24–32 hours both in the fed and in the starved condition.

For the first-instar hoppers the alternative percentages of R.H. used were 65 and 70, and 66 and 100, whereas for the fourth-instar hoppers the alternative humidities were 66 and 100; all these experiments were carried out at 20°C. and at light intensity of 2.1 L.F.L. The first- and the fourth-instar hoppers conditioned at 30 per cent. R.H. and starved remained longer in high humidities than any of the others. This was especially marked in the first instar, and might

be due to loss of water during conditioning and starvation. The first-instar hoppers were more active for a longer period of time, they also hopped and turned more, than those of the fourth instar. The latter walked faster, but their greater speed may be a function of their size.

The loss of weight and the water content of the hoppers after starvation and conditioning was determined, and it became apparent that the loss of weight of starved hoppers conditioned at low humidity was greater than of those conditioned at high R.H. However, the water content after starvation was identical, irrespective of whether the hoppers were conditioned at high or at low humidity. It would be wrong at this stage without further work to speculate on the apparent stability of the water content, but it is worth pointing out that it provides a problem which needs investigation.

Discussion.

In the above work, the hoppers of *S. gregaria* have shown a tendency to stay for longer periods in the higher humidity, provided that it is not beyond 60–70 per cent. R.H. The first-instar hoppers hopped and turned less at about 70 per cent. R.H. and those of the fourth instar at about 60 per cent. R.H. in the conditions of these experiments, and the zone of humidity in which they appeared to be the least agitated has been termed the "zone of minimum activity". Kennedy (1937) found that the hoppers of *Locusta migratoria migratorioides* spent more time in the lower humidities, over all the humidity ranges at 30°C. The hoppers of *S. gregaria* exhibited a different type of behaviour, as up to 60–70 per cent. R.H. they spent more time in the wetter part of the apparatus, but above this range of humidity they showed a reversal of the reaction, i.e., they became more agitated and as a result of the increased activity in the higher humidities spent more time in the drier half of the alternative chamber. The zone of minimum activity of *Schistocerca* hoppers corresponds with the optimum humidity for its development, which, as has been shown by Hamilton (1936, 1950), is from 50 to 70 per cent. R.H. at 32.2°C.

The hoppers of *Schistocerca* either in the desiccated or in both desiccated and starved condition, stay slightly longer in the high humidity than the normally fed hoppers, but they still exhibit the reversal of reaction to humidity beyond the zone of minimum activity. This slight change in the intensity of humidity reaction may be due to loss of water during desiccation and starvation. The effect of starvation with or without accompanying desiccation has also been demonstrated in other insects. Thus the preference for dryness in *Locusta* is weakened on desiccation (Kennedy, 1937).

The time spent in activity is affected by low and high humidity in *Locusta* (Kennedy, 1937), but this was not so in *Schistocerca*, where the duration of activity was significantly affected by light in the first instar, and by light and temperature in the fourth instar. Kennedy (1939, p. 492) came to the conclusion from field observations that *Schistocerca* hoppers were active in high light and inactive in dim light. A number of authors (Bodenheimer & others, 1929, working on *S. gregaria*; Parker, 1930, on *Melanoplus mexicanus* (Sauss.) and *Camnula pellucida* (Scudd.); Hussein, 1937, on *Locusta*, *Schistocerca* and *Nomadacris*; and Kennedy, 1939, on *Schistocerca*) found positive thermokinesis which supports the above finding. The activity also increases on starvation and desiccation, as Ellis (1951) has shown experimentally in *Locusta*. It is suggested by Strel'nikov (1936) that this increase in activity is due to loss of water from the tissues, which increases excitation.

The speed of walking of hoppers is affected by rise in temperature, light and humidity. Key (1936) reports a depressing effect of dry air on the locomotor activity of first-instar hoppers of *Locusta*. He has also found that the older hoppers of *Locusta*, reared very wet, are activated by exposure to dry air, while

those reared in very dry conditions are activated by exposure to wet air. However, he has not taken into consideration light as a stimulating factor. It has been noticed in the present work that increase in light also enhances the speed as well as the time spent active. The rise in temperature correspondingly increases the speed of the hoppers of *Schistocerca*, i.e., they exhibit positive thermo-orthokinesis. The effects of interactions of factors have also been tested in these experiments and it was found that the reactions to combined factors were not always the same as to isolated ones. For example, the interaction of light and temperature significantly affected the behaviour of the first-instar hoppers while the interaction of temperature and humidity and that of light and humidity significantly affected the behaviour of the fourth-instar hoppers. Hence, it can be taken that, in field conditions, the three factors (light, temperature and humidity) are all factors that control the speed of walking of the hoppers. It is interesting that thermoreceptors, i.e., sense organs capable of appreciating degrees of heat, have been recently described by Slifer (1951) in *Locusta migratoria migratorioides*.

It was noted that the first-instar hoppers hop more but march less than the fourth-instar hoppers. This difference in activity has also been observed by Hussein (1937) and Ellis (1951). Hopping activity was increased by both temperature and humidity in the first instar, but it was only affected by humidity in the fourth instar. In *Melanoplus differentialis* (Thos.) (Everly, 1929) and in *Locusta* (Key, 1936), hopping is said to be stimulated by the effects of light, but this does not appear to be so in *Schistocerca* hoppers. Increased hopping activity in dry and in very moist air, and the sudden fall of this form of activity, as well as of the number of turnings, in the zone of minimum activity appears to have no parallel in other similar investigations. It may further be noted that the interaction of temperature and light significantly affects the hopping activity of the fourth-instar hoppers of *Schistocerca*, i.e., the sensory stimulation by these two factors has an effect on the activity of the hoppers.

The turning activity (klinokinesis) increases with the rise of temperature and humidity. The hoppers also respond, by increased turning movements, to the interacting effects of temperature and light. The secondary effect of light is interesting, as it does not influence this type of activity directly. This secondary effect of light is such that the stimulating effect of high temperature is reduced by the higher level of light intensity.

Thus it may be said that the hoppers are more agitated and hop more, show increased orthokinesis and klinokinesis in both low and high humidities. Their activities are reduced at relative humidities of 60–70 per cent.

Summary.

Reactions of 3-day-old first- and fourth-instar hoppers of *Schistocerca gregaria* (Forsk.) of phase *gregaria*, to three physical factors, namely to relative humidity, temperature and light have been studied in an alternative chamber apparatus designed to give an enclosed space with gradients of humidity. The responses to each factor independent of the others, and to the interactions of these factors, were considered.

It was found that both the first- and the fourth-instar hoppers respond strongly to humidity. They are agitated by low, and even more by very high, humidities, but become more quiescent within a zone of 60–70 per cent. R.H., where they spend more time. Within this zone they hop and turn less and this decrease in activity appears to be a response which is independent of both temperature and of light intensity. This zone of decreased agitation has been called "the zone of minimum activity". However, the speed of walking in contrast to the other types of activity, increased progressively with humidity.

Increase in light intensity raises the level of activity at all humidities, *i.e.*, it increases the duration of activity within the limits of experimental time and the speed of walking. With rise in temperature there is an increase in hopping, in klinokinesis and orthokinesis.

The first-instar hoppers respond to the effects of the interactions of light and temperature by increased ortho- and klinokinesis, while the fourth-instar hoppers respond to the interactions of temperature and light by increased hopping. The effects of interactions of temperature and humidity and of light and humidity on the behaviour of the fourth-instar hoppers are also significant.

It was also seen that starved hoppers were generally more active, and that desiccated and starved hoppers spent somewhat more time in the half of the apparatus having the higher relative humidity than did normal, fed hoppers.

Throughout these experiments it was noted that the first-instar hoppers of *Schistocerca* are somewhat more active than those of the fourth instar, and also that the first-instar hoppers hop more, but march less, than those of the fourth instar.

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A CALLIPHORID HOST OF *THYRIDANTHRAX ABRUPTUS*
(LW.) IN NIGERIA (DIPTERA, BOMBYLIIDAE).

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The range of *Thyridanthrax abruptus* (Lw.), a widespread puparial parasite of *Glossina morsitans* Westw. in East Africa, is now extended to British West Africa where this species has been found parasitising a Calliphorid fly, *Rhyncomyia pictifacies* Big. Puparia of *R. pictifacies* were collected in the stream-bed sand of dry-season breeding sites of *Glossina palpalis* (R.-D.) and *G. morsitans submorsitans* Newst. at the following localities in Northern Nigeria: Gamagira, Zaria Province; Mando Road (25 miles NNW. of Kaduna), Zaria Province; Rahama, Bauchi Province. All were collected during January and February 1957. During February, eight adults of *Thyridanthrax* emerged from a total of 33 puparia of *R. pictifacies* found at Rahama.

If *T. abruptus* from Nigeria is indeed conspecific with the species found in East and South Africa, this species cannot be regarded as a specific parasite of *Glossina*. I have not compared male genitalia of the West and East African forms, but Austen's (1929) records of *T. abruptus* from Abyssinia and Natal, where *G. morsitans* does not occur, support this contention; Hesse (1956, p. 573) notes that *T. abruptus* is distributed over southern Africa much more widely than the genus *Glossina*. An early Northern Nigeria record of *T. abruptus* (Marshall in Lamborn, 1915, p. 256) from Minna is considered a misidentification by Austen (1929, p. 158) but the specimen, a female in the British Museum collection, might bear re-examining.

Thyridanthrax argentifrons Aust. is as yet the only species of this genus known to parasitise *Glossina* in Nigeria. Taylor (1932, p. 466), at Gadau in Bauchi Province, records parasitism rates of 0.66 per cent. among 106,047 puparia of *G. tachinoides* Westw. and 0.27 per cent. among 17,846 puparia of *G. m. submorsitans*.

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A STUDY OF THE AGE OF FEMALES OF *SIMULIUM ORNATUM* MG. (DIPTERA) ATTRACTED TO CATTLE.

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L. Davies (1957) has recorded the effect of external conditions on the landing activity of *Simulium ornatum* Mg. (Diptera, SIMULIIDAE) on cattle. The present paper describes studies undertaken to determine the age composition of catches of this blackfly attracted to cattle in the field. Using the system of age classification referred to by L. Davies (1955) and described in detail below, it has been possible to investigate variations in the age composition of such fly-catches during part of one season, and also during short periods, such as one day, when important variations were detected and occurred with some regularity. A study of the events on certain days when the normal short-term variation in age composition of the catch failed to occur was made with a view to elucidating the complex of factors responsible for such rapid age-composition changes.

Methods.

Collection of blackflies off cattle.

The cattle used were barren Shorthorn and Ayrshire heifers 1½-2 yr. old which were not tethered and grazed a pasture some 15 acres in extent situated 5 km. east of Durham, England (lat. 54° 46' N) during 1952-54. The cattle were habituated to the presence and activity of the collector, who devoted attention to one cow at a time. Since females of *S. ornatum* landed exclusively on the ventral surfaces of the cow, it was possible to collect almost all flies as they landed, negligible numbers being lost by disturbance before they could be sucked into a "pooter" of the pattern already described (L. Davies, 1957). The age composition was thus not seriously biased by the loss of flies of a particular age-group. At the end of a fly-collection period of 10-60 min. the flies in the "pooter" were killed with ethyl acetate vapour and fixed in 70 per cent. ethyl alcohol.

Laboratory maintenance of blackflies.

In order to study changes in flies with increasing age, females of *S. ornatum* were kept in the laboratory in 2 × 1 in. tubes, as described by Lewis (1953), closed by mosquito netting and containing a circle of filter paper in the bottom and an upright strip of the same paper to allow the fly to walk readily to the top. The tubes were stored at approximately 100 per cent. R.H. and flies were kept for periods of three weeks with low mortality provided the temperature was kept sufficiently constant to prevent condensation occurring within the vessels. Sucrose, readily taken by flies, was provided on small pieces of cotton-wool placed on the netting closing each tube, the cotton-wool with absorbed sugar solution being renewed every two days. After a fly had been kept for the desired number of days it was killed and preserved in ethyl alcohol to await dissection.

Dissection of blackfly females.

Flies were dissected in a waxed dish under a binocular microscope at × 31.25 magnification. Each specimen was pinned ventral-side-up with a fine steel pin through the thorax. It was possible to remove the hind legs and open the

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abdomen by simply pulling the hind coxae backwards with forceps. This not only detached the legs but at the same time removed a strip of ventral abdominal cuticle. The abdomen was further opened with fine needles and the contents examined. Fixation rendered the fat-body cells opaque and white so that the amount of fat-body was more easily evaluated in preserved flies. In fresh flies, the fat-body was largely transparent and thus more difficult to see. Therefore, since it was eventually decided to base the age classification of field-caught flies mainly on the state of the fat-body, dissections were mainly done on alcohol-preserved flies, although some were dissected in a fresh condition to check results obtained with preserved individuals.

Age-dependent Changes in Females of *S. ornatum*.

Non-blood-fed flies.

These were hatched from pupae collected from the stream alongside the cattle pasture (L. Davies, 1957). Some were killed and dissected on emergence and others were kept alive in the laboratory. Two series were maintained, (1) in darkness at 16–18°C., and (2) subjected to approximately 9 hr. artificial light per day at 22–23°C. The age of these adults from pupae was known, and changes occurring with increasing age were investigated by dissection and external examination.

Flies killed within one day of emergence from the pupa invariably possessed abundant fat-body consisting of large masses particularly conspicuous in the anterior abdomen. These masses largely obscured and enveloped the ventral

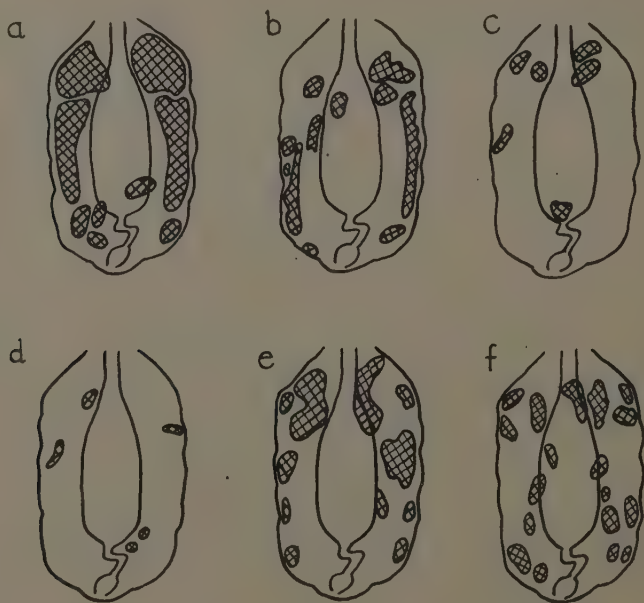


Fig. 1.—Amount of fat-body (cross hatched) in non-blood-fed flies of different ages. a, freshly emerged fly; b, 7-day old, 22–23°C.; c, d, 14-day old, 22–23°C.; e, 7-day old, 16–18°C.; f, 14-day old, 16–18°C.

aspect of the gut, ovaries and Malpighian tubes and occupied about 25-35 per cent. of the abdominal volume. The crop was always empty or nearly so.

With increasing age the outstanding internal change that occurred was the comparatively rapid reduction in the amount of fat-body, which after 14 days at 22-23°C. consisted of small flattened masses scattered in various parts of the abdomen. Drawings of representative specimens illustrating these changes in the amount of fat-body are given in fig. 1. Fat-body disappeared more rapidly in flies kept at 22-23°C. than in those at 16-18°C., as would be expected, although considerable variation occurred between individuals kept at the same temperature. A fly kept at 16-18°C. for 14 days usually reached the same stage of fat-body depletion as one kept at 22-23° for about 7 days. In flies older than about 2-3 days, the crop was usually distended with liquid, and in flies older than 7 days, the distended crop filled a large proportion of the abdominal volume, occupying the space previously taken by the fat-body in freshly emerged flies. This distension of the crop indicates that the flies fed readily on the sucrose solution, the only liquid available to them in the vessels in which they were kept.

The effect of fat-body depletion on the dry-matter content of flies was determined on a series of females obtained from the same batch of pupae and dried to constant weight at 50 per cent. R.H. at room temperature. The results, summarised in Table I, giving the mean dry weight of the abdomina of 14-day-old

TABLE I.

Dry weights of whole females and abdomina (mg.) of *S. ornatum*.

		0-day-old flies	14-day-old flies
Whole flies (20)	Range	0.850 — 1.250	—
	Mean and S.D.	1.087 ± 0.105	—
Abdomina (10)	Range	0.323 — 0.485	0.203 — 0.305
	Mean and S.D.	0.369 ± 0.049	0.237 ± 0.025

and of freshly emerged females, show that a loss of about 36 per cent. in the dry weight had occurred in 14 days. Since the abdomen of freshly emerged flies represented about 33 per cent. of the total dry body weight, a 36 per cent. loss in abdominal weights amounts to a loss of about 13 per cent. of the total dry body weight. This loss is probably largely a reflection of the depletion of matter stored in the fat-body.

Concurrent with the depletion of fat-body there occurred in non-blood-fed females a noticeable increase in the volume of the ovaries, and the length of the lowest oöcytes in the ovarioles increased from a mean of 65 microns on emergence to 91 microns on the fifth day, after which no further increase occurred (fig. 2). This growth of oöcytes was accomplished presumably at the expense of fat-body reserves and agrees with the observations of Wanson & Lebiec (1948) on *S. damnosum* Theo. In *S. ornatum*, further growth of the oöcytes to full maturity occurred only in flies engorged on blood (see below).

Organs other than the fat-body were examined in flies of known age to see whether they afforded means of approximate age determination. Changes in the opacity of the haltere knob, described by Lewis (1953) for *S. damnosum*, appeared to be so much less marked in *S. ornatum* as to be of little use for age determination even in fresh flies. Changes in the colour of the Malpighian tubes recorded for *S. venustum* Say by Pomeroy (1916) occurred in *S. ornatum* also, but did not

seem to be correlated with age. Other features examined were wing-fraying and the amount of hair on the dorsum and pleural membrane of the thorax. In flies taken in the field, where the presence of relict ripe eggs proved that they had completed a gonotrophic cycle and were thus old, neither loss of thoracic hair nor wing-fraying was observed. Wing-fraying, but not loss of hair, did occur in groups of flies confined in jars in the laboratory for 14 days and exposed to 9 hr. light per day.

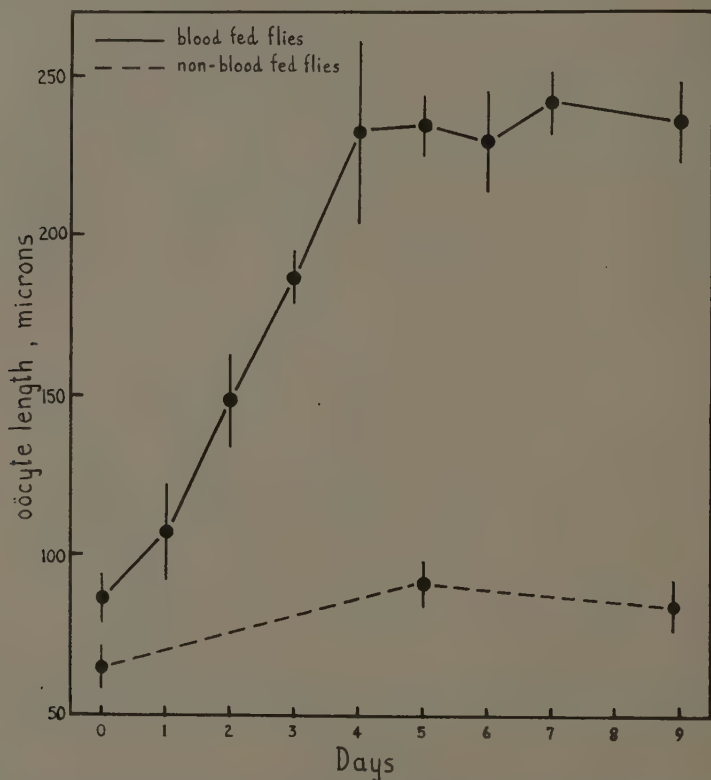


Fig. 2.—Mean oöcyte length in blood-fed and non-blood-fed females. Each point is the mean of 35–75 eggs from 3–5 individuals. Vertical lines show standard deviations.

In laboratory flies more than about ten days old, the abdominal epidermis seen from the inner surface was sometimes reddish-brown in colour in contrast to the grey colour found in younger flies. This colour change may have been a fixation artefact and since it did not invariably occur in older flies it could not be used for age determination.

Details are given, in Table II, of the survival of flies of the 16–18°C. series in the laboratory. Flies fed on sucrose solution suffered only 6 per cent. mortality in 14 days. The value of sucrose in prolonging survival is seen by the reduced

length of life of those fed on distilled water, and the even shorter survival of those not fed at all indicates that water prolonged survival for a few days. In 10 groups, each of 20 females, kept at 22–23°C. and fed on sucrose, the mortality within 5–14 days was 10–25 per cent. except in three groups where 50–75 per cent. of the flies died within that period, the high mortality being caused in each case by the trapping of flies in condensed moisture within the jars.

TABLE II.

Mortality of flies maintained in the laboratory at 16–18°C.

Fly category	Treatment	No. flies	Fate
Non-blood-fed	Fed sucrose	125	6% dead after 14 days
Non-blood-fed	Fed dist. water	30	70% dead after 7 days
Non-blood-fed	Not fed	15	All dead in 5 days
Blood-fed	Fed sucrose	25	8% dead in 1–7 days
Blood-fed	„	52	20% dead in 8–19 days

Blood-fed flies.

These were obtained from grazing cattle as explained below. In the course of three seasons' work, flies which were observed to become attached to a cow invariably became fully engorged unless killed by the action of the cow's tongue (L. Davies, 1957). There was no evidence that partial blood-feeds occurred at all in *S. ornatum* feeding on cattle. On becoming engorged in the field the flies detached themselves and took to flight extremely suddenly before they could be caught. When a fly approached full engorgement, as judged by the extreme distension of the abdomen, it was gently pushed with the inside of the edge of a 2 × 1 in. glass tube containing a filter paper circle and strip. If almost engorged, the fly usually detached itself and fell into the tube which was quickly covered with mosquito netting. Flies obtained in this way had probably ingested slightly less than the normal full amount taken on engorgement, and were kept in the tubes at approximately 100 per cent. R.H., at 16–18°C. and provided with sucrose solution. They suffered very little mortality up to 19 days (Table II). It is clear from these results and from those of Steward (1937) and Gnedina (1950), who kept engorged females of *S. ornatum* for up to 30 days, that this species survives far better in the laboratory than do certain other blackflies, such as *S. venustum* and others, in which Wu (1931) and D. M. Davies (1953) record heavy mortality within one week.

In *S. ornatum*, during the first 12 hr. after engorgement, the abdomen gradually returned to its normal undistended state, presumably because of reduction in the volume of the blood-meal by elimination of excess fluid in the faeces which were produced in the form of a brown liquid during this time. Thirty five flies were dissected 0–3 days after engorgement and in every case blood was present in the expanded posterior region of the mid-gut, never in the crop, agreeing with the results of Blacklock (1926) and Lewis (1953) for *S. damnosum*. A peritrophic membrane was clearly visible in these flies and its structure agreed with that of *S. damnosum* described by Lewis. Changes in the appearance of the blood-meal during digestion in *S. ornatum* agreed with the information for *S. damnosum* given by Wanson & Lebiec (1948). In ten specimens dissected four days after a

blood-feed, eight had a completely empty mid-gut apart from the remains of the peritrophic membrane, while the other two still contained a small amount of the much changed remains of the blood-meal.

The mean lengths of the oöcytes of flies at various intervals after engorgement (fig. 2) show that appreciable increase in length had occurred after two days and that they reached a maximum in four days, after which no further significant increase in oöcyte length had occurred in flies killed 5-9 days after the blood-meal. The mean oöcyte length of around 230 microns in flies killed four or more days after engorging (fig. 2) is appreciably lower than the mean length of 285 microns observed for laid eggs of *S. ornatum* collected in the field. The method used to collect blood-fed flies from cattle probably involved curtailing ovary development because of shortage of nutrient derived from the blood-meal, leading to a smaller final egg size as compared with eggs laid in the field. Alternatively, eggs of *S. ornatum* may swell slightly after laying. It is concluded that blood-fed females of *S. ornatum* at 16-18°C. became fully gravid 4-5 days after engorgement. In 40 flies, examined four or more days after engorgement, the ovaries in every individual were packed with fully shelled eggs, and all flies could be classed as fully gravid. The ovaries occupied most of the volume of the abdomen and the gut was markedly compressed between them. It is concluded that a blood-meal in females of *S. ornatum* invariably led to egg development. In view of the failure of eggs to mature in non-blood-fed flies treated similarly to blood-fed flies, it seems that a blood-meal is necessary before ripe eggs are produced.

All flies killed four or more days after feeding on blood were closely examined for traces of fat-body. Out of 40 individuals, no trace of fat-body could be found in 36, and in the remaining four the fat-body was reduced to minute traces in the posterior dorsal region of the abdomen. Some of these flies would be engorging for the first time and would have contained visible fat-body at that time, since that tissue was often readily detectable in freshly engorged individuals. It seems that blood-fed flies which later reached the fully gravid stage then contained no visible fat-body except possibly in small amounts in the posterior abdominal segments. Any fat-body present on engorging was used up during egg development, and there was no evidence that the products of digesting a blood-meal could be used to build up fat-body.

Lewis (1953) described the existence in *S. damnosum* of the shrunken remains of the peritrophic membrane after digestion of the blood-meal is complete, and he was able to use its presence as evidence of a previous engorgement. In *S. ornatum*, remains of the peritrophic membrane was seen on occasions, but it was too difficult to detect at the magnification used in the present work for routine dissection to be used as an age indicator. Further, in two engorged flies kept alive for 23 days, remains of the peritrophic membrane could not be found even when the gut was examined at $\times 100$. Presumably it may be lost by the fly after a considerable time has elapsed.

The remarks concerning the colour of the abdominal epidermis in non-blood-fed flies (page 538) applied also to blood-fed individuals.

Internal Condition of Wild-caught Flies.

The crop, in unengorged flies taken in the field, was distended and usually contained some gritty particles similar to those described by Lewis in *S. damnosum*. No definite pollen grains were detected, but frequently fungal spores were present, sometimes in such large amounts as to render the contents of the crop milky white in colour. In some specimens a milky white appearance of the contents was imparted by very fine unidentified particles. Black gritty particles similar to those in the crop were usually present in the mid-gut and sometimes in the hind gut.

It was found that wild-caught flies could be divided into two main categories

on the basis of the amount of fat-body present, hereafter referred to as "A" and "B" flies.

"A" flies.—These contained visible fat-body, usually conspicuous in the anterior end of the abdomen. The crop occupied a proportion of the volume of the abdomen inversely proportional to the amount of fat-body. "A" flies seemed to form a graduated series with decreasing fat-body, corresponding in appearance to fig. 1, b-d. Depletion of fat-body in laboratory flies with increasing age suggests that the less fat-body present in the wild-caught flies, the greater was their probable age. Since very few "A" flies from cattle contained as much fat-body as was observed in flies freshly emerged from pupae (fig. 1, a) it seems that the very young flies, about 1-2 days old, were not attracted to the cattle. Presumably during the first two days or so of adult life, mating and dispersion of the flies from the stream occurred.

Relict ripe eggs were not found in "A" flies except in two exceptional cases which are discussed on page 542.

"B" flies.—The criterion adopted in classifying flies as "B" flies was the complete absence of detectable fat-body in the anterior abdomen. Most of these flies contained no visible fat-body whatsoever, while, in the remainder, small rounded cell groups in the posterior abdominal region were all that remained of it. Apart from the distended crop which usually filled most of the abdomen, the latter was remarkably empty, with the gut devoid of contents apart from small gritty particles. The ovaries were sometimes highly compressed and in other individuals not so. The abdominal epidermis was usually reddish brown in colour as compared with the grey colour found in most "A" flies. On average, about 16 per cent. of the flies so classed contained one or more relict ripe eggs. It is likely that most flies would on oviposition lay all their eggs and only a minority would retain a small number, thus providing evidence of a previous gonotrophic cycle when subsequently captured. This is considered to be the case in mosquitoes generally (Bates, 1949, p. 91). In *Anopheles sacharovi* Favr, Mer (1932) found relict eggs in only 10 per cent. of females known to have oviposited. The occurrence of relict eggs in 16 per cent. of "B" flies thus, it is suggested, indicates that a large proportion, probably most of them, had previously fed on blood and undergone at least one complete gonotrophic cycle. The absence of fat-body in "B" flies generally confirms this in view of the observations on blood-fed flies that reached the fully gravid stage in the laboratory.

As an approximation it is therefore considered that dividing wild-caught flies into "A" and "B" categories on the visible fat-body basis separated most of the flies which had not obtained a blood-meal (A) from those that had done so (B). In the absence of evidence of partial blood-feeds in the field, and the invariable development of mature eggs in blood-fed flies kept in the laboratory, it is considered unlikely that many "A" flies had obtained blood and failed to mature their eggs. A source of inaccuracy in the separation of non-blood-fed and blood-fed individuals by the above criterion of presence or absence of fat-body would be the occurrence of flies in the field which had reached such an age as to have no visible fat-body but had not taken a blood-meal. In the study area, where never less than 40 cattle grazed within 0.5 km. of the stream providing the main source of breeding throughout the blackfly season, it seems probable that the density of the cattle would be sufficient to give a high chance of most flies finding a host to bite before their fat-body had virtually disappeared to the "B" condition. Assuming that fat-body depletion occurred in field flies at the same rate as in laboratory flies kept at 16-18°C., such flies would have to be over 14 days old before they alighted on the cow. It is unlikely that individuals with this history formed an important part of the flies collected from the cattle.

Two further possible sources of departure of "A" or "B" categories from correspondence with the non-blood-fed and blood-fed groups, respectively, remain:—

1. The completion of a gonotrophic cycle by some individuals while visible fat-body remained in the anterior abdomen.

2. The rebuilding of fat-body, in flies that had already oviposited, possibly by feeding on carbohydrate such as nectar or honeydew. Such flies might thus return to a state which on dissection would simulate that of young flies that had never depleted the abundant fat-body present on emergence from the pupa.

If either or both of these possibilities occurred with any frequency in the field, one would expect relict eggs to be frequently encountered in "A" flies, *i.e.*, those with readily detectable fat-body. This, however, was not the case, and it shows that possibilities 1 and 2 must occur only rarely in the field. For, in 3,216 "A" flies, relict eggs were found in only 2 cases, *i.e.*, in 0.06 per cent. whilst amongst 1,886 "B" flies, 311 (= 16.5%) contained relict eggs. That is, relict eggs occurred about 275 times more frequently in "B" flies than in "A".

A small proportion, varying from 0–10 per cent. of the total catch, could not be ascribed to either the "A" or "B" category because its was not possible to be certain whether any fat-body was present in the anterior abdomen, and they were regarded as unclassified. They were probably intermediate in character between "A" and "B" flies, although some may have been unclassifiable because of bad fixation.

TABLE III.

Frequency distribution of number of relict eggs per female.

No. of eggs	Frequency in flies taken in :	
	1952–53	1954
1	82	165
2	20	54
3	9	25
4	6	12
5	5	4
6	5	1
7	3	4
8	5	3
9	2	1
10	1	1
11	0	1
12	3	1
13	1	1
14–20	0	3
> 20	10	32

Presence of relict eggs in wild-caught flies.

When present, relict eggs were usually situated either in the ovary or in the oviducts, but were sometimes found quite outside the ovary, *e.g.*, in the extreme anterior end of the abdomen, having presumably broken out of the ovary when the fly was gravid. The frequency distribution of the number of relict eggs per female is shown in Table III. It was possible that this distribution might correspond with a known type of distribution for which the zero term could be calculated. If this were so, an independent estimate would be obtained of the number of flies which had previously undergone a gonotrophic cycle but had laid all their ripe eggs. This calculated figure could be compared with the figure obtained by the fat-body classification into "B" flies. It seems, however, that the zero term of the series cannot be calculated with any useful degree of accuracy.

Age Composition of S. ornatum from Cattle.

All times in the following account refer to Greenwich Mean Time.

Flies taken in 1952-53.

In 1952, all blackflies taken on cattle were pinned to ensure their correct identification, since the occurrence of species other than *S. ornatum* had to be investigated. The catches made on certain days chosen at random were soaked in 25 per cent. ethyl alcohol overnight, and then in a 1 per cent. sodium sulphite solution for 3 hr. to soften and swell the contents of each fly further. The abdomina of the flies were then opened and the presence of relict ripe eggs in the otherwise liquefied abdominal contents proved readily detectable. The number of flies containing such eggs in the pooled results for 11 days are given in Table IV (a). The proportion of flies containing relict eggs was greater in those taken before 9 a.m. than in those taken between 9 a.m. and 6 p.m. These figures suggest that on the days in question the proportion of old flies was on average appreciably greater in early morning than during later morning and afternoon.

TABLE IV.

(a) Frequency of flies containing relict eggs, 1952.

Flies taken 5-9 a.m.		Flies taken 9 a.m.-6 p.m.		χ^2	P
No. with relict eggs	No. of flies	No. with relict eggs	No. of flies		
18 (=18.7%)	96	5 (=4.5%)	111	9.18	< .01

(b) Frequency of flies classed as old, 1953.

Period	No. of flies	No. old	% total flies classed as old	
6-9 a.m. ..	51	26	51.0	$\chi^2 = 4.72$
9 a.m. - 6 p.m.	715	325	45.4	P = .10
6-9 p.m. . . .	277	155	55.9	
6-8 p.m. ..	133	61	45.8	$\chi^2 = 9.80$
8-9 p.m. ..	144	94	65.2	P = < .01

In 1953, blackflies taken on cattle were preserved in ethyl alcohol and dissected by the method earlier described. The results (Table IV, b) show that although higher proportions of old flies were taken from 6 to 9 a.m. and from 6 to 9 p.m. than from 9 a.m. to 6 p.m. the differences were not statistically significant. When, however, the 6 to 9 p.m. flies are divided into those taken from 6 to 8 p.m. and 8 to 9 p.m. (bottom part of Table IV, b) it is seen that a significantly greater proportion ($P = < .01$) of old flies was taken in the latter period, which corresponded approximately to the hour before dusk.

Seasonal changes in the proportion of old flies, 1954.

An index of the changing abundance of *S. ornatum* on a cow during part of the 1954 season was obtained as follows. The mean maximum number of flies landing on a cow per 15 min. during the last hour of daylight on calm evenings was calculated for each of five parts of the season. The number of flies landing on a cow is likely to be more closely related to fly abundance in late evening than during the day when the inhibitory effects of wind or too great an evaporation rate are likely to mask seasonal effects. Only data obtained on calm evenings were used. In a previous paper (L. Davies, 1957) the above indices of fly abundance on cattle were used to correct figures of the number of flies landing under different humidity conditions for variation in fly abundance. Comparison of corrected and uncorrected figures obtained from the two halves of the 1954 season afforded evidence that the indices did provide a reasonably accurate picture of changes, during the season, in the abundance of *S. ornatum* landing on cattle (L. Davies, 1957, fig. 3).

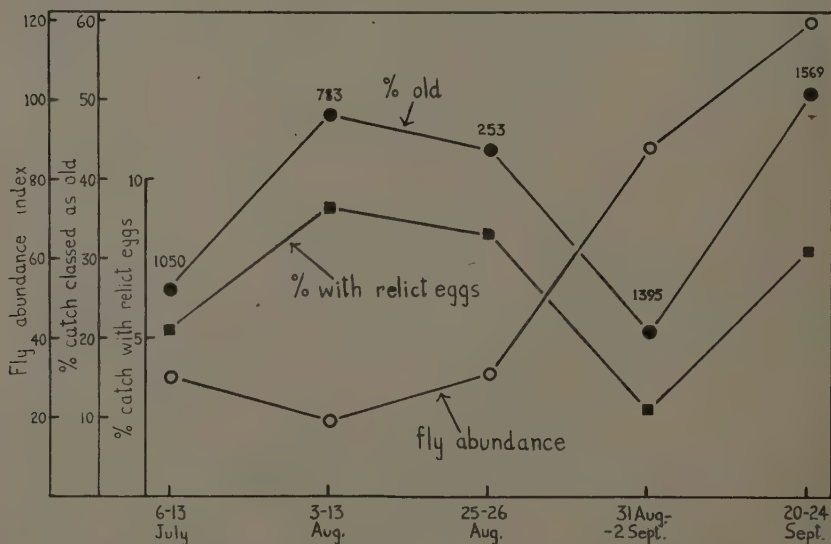


Fig. 3.—Seasonal variation in fly abundance and in the age composition of the catch. Numbers above points give the number of flies taken and dissected.

In fig. 3, the fly abundance index for the five parts of the 1954 season is graphed together with the proportion of old flies and the percentage containing relict eggs in the totals of those taken during the same five periods. The

proportion which contained relict eggs among the total flies caught fluctuated in a manner parallel to changes in the proportion of flies classified as old (B flies) on the basis of the amount of fat-body. The former provides independent confirmation of changes in the latter. The changes in the proportion of old flies between 6th-13th July and 31st August-2nd September (fig. 3) tended to be inversely related to fly abundance changes, as would be expected on theoretical grounds. In contrast, between 31st August-2nd September and 20th-24th September it is seen that the proportion of old flies increased at the same time as fly abundance increased.

The increase in proportion of old flies from early to late September is well substantiated, since the result obtained by fat-body classification is confirmed by the increase in the proportion containing relict eggs in the total catch, in the same period. Increase in the abundance of flies landing on cattle during the same period seems equally well established, as shown by the following figures. The mean maximum number of flies landing per 15 min. in late evening (= Fly abundance index in fig. 3) increased from 87 to 119 (increase of 36%) and the mean number of flies landing per 15 min. throughout the day rose from 35 (mean of 47 observations) in early September to 49 (mean of 35 observations) in late September, an increase of 40 per cent.

TABLE V.

Proportion of old flies for periods ranging from 1 to 3 hours at varying times between 10 a.m. and 6 p.m. as compared with the last $\frac{1}{2}$ to $1\frac{1}{2}$ hours of daylight.

Date	10 a.m.—6 p.m.		6—9 p.m.			χ^2	P
	Period	No. flies	No. old	Period	No. flies	No. old	
7 July	11.00—1.00	108	10	8.00—8.30	112	42	22.75 < .001
8 "	10—11, 12—1	99	10	8.00—8.45	79	20	6.01 < .02
9 "	4.30—5.00	32	3	8.00—8.50	86	33	—
12 "	4.20—5.00	61	16	8.15—9.00	40	29	19.09 < .001
13 "	12.20—3.00	58	16	8.15—9.00	59	34	9.58 < .01
3 Aug.	12.00—4.00	64	19	7.00—8.30	94	79	45.48 < .001
7 "	1.00—3.00	65	12	7.00—8.25	83	30	4.77 < .05
12 "	10.00—11.00	41	9	7.00—8.00	86	55	17.94 < .001
13 "	3.30—6.00	43	16	7.00—8.15	67	23	0.01 c. .90
25 "	3.00—6.00	61	22	6.00—7.30	55	25	0.70 c. .50
26 "	11.30—2.00	68	16	7.00—7.45	69	47	25.63 < .001
31 "	4.30—6.00	70	11	6.30—7.20	79	17	0.48 .50
1 Sept.	2.20—2.50	95	13	6.25—6.55	95	41	18.68 < .001
2 "	2.10—3.40	102	10	6.30—7.00	75	29	19.30 < .001
21 "	2.50—3.40	103	52	5.40—6.10	126	86	6.74 < .01
23 "	2.40—3.30	66	22	5.20—5.50	34	10	0.03 c. .90
Totals		1136	257		1239	600	19.68 < .001
No. containing relict eggs in totals		41			123		31.82 < .001

Diurnal changes in the proportion of old flies, 1954.

In Table V the proportion of old flies landing on one cow for periods ranging from 1 to 3 hours at varying times between 10 a.m. to 6 p.m. is compared with the proportion of old flies in catches made in the last $\frac{1}{2}$ – $1\frac{1}{2}$ hr. of daylight. In the pooled results for the 16 days on which records were made, a significantly higher proportion of old flies was present in the late-evening catches. When the

16 days are considered separately and the χ^2 test is applied to the results for 15 of the days (Table V) a statistically significant increase ($P = < .001$) in the proportion of old flies landing in late evening occurred on 7 days. From Table V it can be calculated that in the total day-time catching periods of 28 hr., 257 old flies landed, while in the total late-evening periods of 13.75 hr., 600 old flies landed on the cow. These figures give a mean number of old flies landing per 15 min. of 2.3 during the day-time and 10.9 in late evening. On the 16 days, therefore, the mean number of old flies landing per unit time was 4.5 times greater in late evening than during the day.

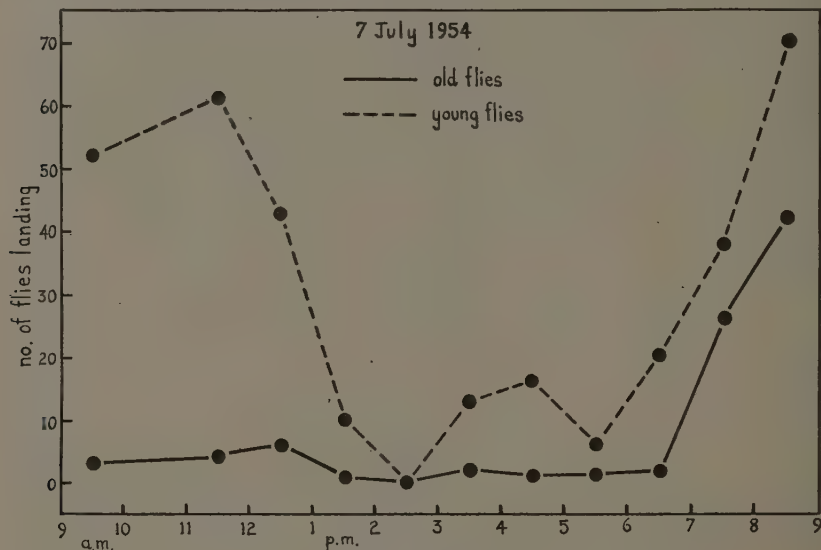


Fig. 4.—Changes in numbers of young and old flies landing on a cow, 7th July 1954.

More light is thrown on these short-term changes in the proportion of old flies in the catch by examining the results of dissecting the whole catch obtained from early morning to dusk on certain days. On 7th July (fig. 4) the number of young flies fluctuated greatly throughout the day, while the number of old flies remained remarkably constant from 9 a.m. to 7 p.m. In late evening, the numbers of both young and old flies increased steeply. It seems that factors or combinations thereof that stimulated fly-landing activity during the day, tended to stimulate only the young flies and did not affect the old flies, while in late evening the landing activity of both groups was stimulated. Similar results were obtained on 1st and 2nd September, the only other days for which all flies taken throughout the day have been dissected, and can be summarised by saying that during the day large fluctuations in the number of young flies occurred, with numbers of old flies remaining constant at a rather low level, while in late evening the numbers of both old and young flies usually fluctuated in unison.

Information on changes in the numbers of old and young flies is available for parts of the period 20th–23rd September and representative parts are given in fig. 5. It will be seen that here the number of old and young flies showed large and closely parallel fluctuations. It seems that the landing activity of young

flies was stimulated or inhibited to a degree similar to that of old flies, and that, on autumn days, old and young flies behaved in a manner which was only detected in late evening on days earlier in the season.

On 4 of the 16 days listed in Table V, namely, 13th, 25th, 31st August and 23rd September, no increase in the proportion of old flies occurred in late

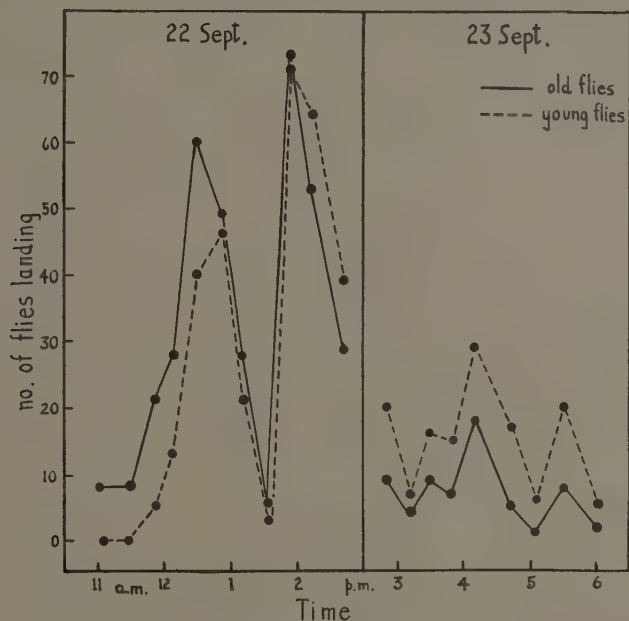


Fig. 5.—Changes in numbers of young and old flies landing on a cow, 22nd, 23rd September 1954.

evening. On the remaining 12 days such an increase occurred although it was statistically significant in 7 cases only. It is instructive to compare the fly-landing activity pattern of evenings on which an increase in the proportion of old flies did not occur, with the pattern during corresponding periods on adjacent days, when such an increase took place.

On 12th August, fly activity remained heavy right up till darkness fell at 8 p.m. (fig. 6a). On this evening there was low cloud, and this accounts for the early onset of darkness, and probably for the fact that the air temperature fell very slowly and was still 12.5°C. at 8 p.m. On 13th August, a cloudless evening (fig. 6b), landing activity was far less from 7–8 p.m. than on the previous evening and was very slight after 8 p.m., ceasing by 8.15 p.m. in spite of the fact that darkness did not fall until 8.30 p.m. It seems that, on 13th August, late-evening fly activity was curtailed, possibly because of the rapid fall in air temperature. Whatever environmental factors were responsible for this curtailment on 13th August, they seem to have in effect led to the omission or considerable reduction of late-evening fly-landing activity when old flies normally tended to be particularly active.

Similar differences seem to have occurred between the two evenings 25th and 26th August (fig. 6, d & e). As on 13th August, the curtailment of activity in

the late evening of 25th August, again possibly caused by the rapid temperature fall, appears to have largely eliminated the phase characterised by increased activity of old flies.

On 31st August and 1st September, late-evening fly-landing activity occurred on a considerable scale until darkness fell. On 31st August, however, no late-evening increase in the proportion of old flies occurred, while a considerable increase in old flies occurred on the evening of 1st September (Table V). On

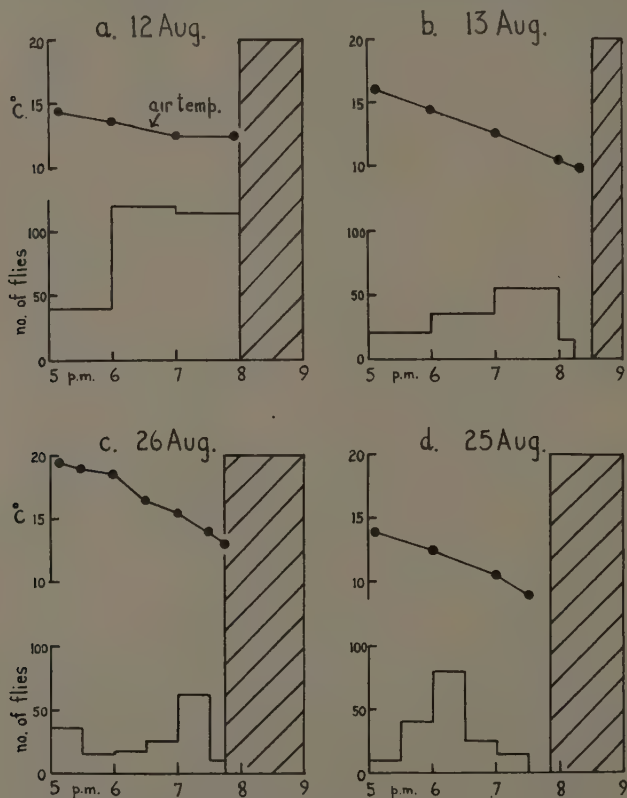


Fig. 6.—Comparison of late-evening fly activity on a cow on two pairs of adjacent evenings. The cross-hatched area to the right of each figure represents darkness.

both evenings air temperatures throughout exceeded 15°C.; but on 31st August a constant strong breeze occurred, while on 1st September the evening was calm. L. Davies (1957) showed that a breeze of 5 m.p.h. was sufficient to reduce landing activity of *S. ornatum* on cattle considerably. The breeze on 31st August depressed activity by approximately one half, as is shown by comparing the number of flies landing on a cow during the last two consecutive hours of daylight. These were:—61 and 95 on 31st August and 120 and 196 on 1st September. The depression of landing activity on 31st August, caused by the

breeze, apparently affected old and young flies differentially, the numbers of the former being reduced to a greater extent than of the latter, so that the proportion of old flies in late evening on 31st August failed to increase as it did on 1st September when conditions were calm.

Readiness of young and old flies to bite a cow.

The readiness of young and old flies to bite a cow was compared by recording the frequency at which fresh blood was found in the gut of flies of each group, which had been given the opportunity to bite during about 10 min. of each hour of fly-collecting periods. The results of dissecting 3,702 flies taken on 18 days in 1954 (Table VI) show that, on average over the period, old flies bit the cow almost twice as frequently as did young flies. When the results are split into three 6-day periods, however, a significantly greater frequency of biting in old flies occurred only in the third period.

TABLE VI.

Frequency of biting in young and old flies taken on 18 days in 1954.

Period	Young flies		Old flies		χ^2	P
	No. taken	No. with blood in mid-gut	No. taken	No. with blood in mid-gut		
1st 6 days	730	32 (4.4%)	280	13 (4.6%)	—	—
2nd 6 days	349	10 (2.9%)	268	17 (6.3%)	3.59	c. .05
3rd 6 days	1369	38 (2.8%)	706	50 (7.1%)	20.22	< .001
18 days summed	2448	80 (3.32%)	1254	80 (6.4%)	18.67	< .001

Discussion.

The presence of large amounts of fat-body in females of *S. ornatum* on emergence from the pupa, and its reduction with increasing age show that in these respects the species is similar to *S. damnosum*, studied by Lewis (1953), and to the various blackfly species studied by Rubtsov (1940). These changes in the amount of food reserves in blackflies appear to form the converse of the situation found in male tsetse flies (Buxton & Lewis, 1934; Jackson, 1937) where the fly on emergence from the pupa contains small reserves which may be augmented considerably by the synthesis of fat after blood-feeds have been taken. In *S. ornatum* there was no evidence that fat-body reserves were built up either from blood or other ingested material such as nectar or honeydew.

In 5,050 dissections of females of *S. ornatum* taken on one cow in 1954, 1,870 or 37.0 per cent. were classed as having already undergone at least one complete gonotrophic cycle. At present it does not seem possible to calculate from this figure the mortality occurring between the young and old fly stage. The lengths of time during which a fly living under natural conditions in the field would have been classed as young or old are unknown. Furthermore, the average of 37.0 per cent. old flies over the season covered periods of both apparent increase and decrease of abundance, probably leading to a complex relationship between the proportions of the two groups of flies at any given time.

The changes in the proportion of old flies landing on a cow during 1954 (fig. 3) show that during mid-season this proportion was inversely related to fly

abundance, as would be expected, but that in early autumn this was not the case. These results suggest that length of life of the flies must have been considerably greater in late September than earlier in the season, or that some seasonal change occurred in the reaction of flies of different ages to the cattle.

In 1952, a higher proportion of flies taken on cattle were found to contain relict eggs in early morning than during the middle of the day. This result is based on relatively few flies and cannot be considered as adequately established. In 1954, a statistically significant increase in the proportions of old flies landing on the cow in late evening as compared with during the day was found on several occasions. D. M. Davies (1952) has shown that the activity of *S. venustum* is markedly influenced by those factors that influence the evaporation rate, notably temperature, humidity and wind speed. The latter factor has been shown to have a great effect on the number of females of *S. ornatum* landing on cattle (L. Davies, 1957). The increased activity of old females of *S. ornatum* in late evening and possibly in early morning, found in the present work, broadly coincided with the occurrence, at those times of day, of less rigorous environmental conditions, namely lower temperatures, higher humidities and lower wind speeds.

The rapid changes in the course of one day in the age composition of flies landing on cattle suggest that either young and old flies reacted to different threshold values of the environmental factors controlling activity, or that flies had similar thresholds regardless of age, but that the changing environmental factors affected differentially the proportion of old flies, as compared with young flies, that succeeded in arriving at the cow. In the first alternative, environmental factors would possibly produce their effects by operating on the flies at the time at which they commenced to become active and left their resting sites. In the second case these factors possibly operated during the time when they were on the wing seeking a suitable host. The second hypothesis presupposes that old flies were more susceptible than young flies to some detrimental factors such as high evaporation rates, a state of affairs suggested by the apparently more adverse effect of wind on old flies (p. 549). The fact that, on typical days in mid-season, the number of old flies landing often remained remarkably steady while large fluctuations in the numbers of young flies occurred (fig. 4), suggests that old and young flies react differently under the same conditions. The peaks in the number of young flies seem to have been due to a stimulatory situation which hardly affected the old flies at all. On the other hand, in late evening in mid-season and throughout daylight in late season the numbers of old and young flies were usually closely correlated, suggesting that unequal success of old and young flies in reaching the cow largely produced the rapid changes in age composition during the day in mid-season.

The greater relative frequency of fresh blood in the gut of old flies than of young flies at certain times shows that the former were in effect more ready to bite the cow on some days, or possibly that, under the collection system used, old flies showed more speed in starting to bite than did young individuals. This difference may reflect the greater "hunger" of old flies in so far as the concept of hunger can be applied to an insect.

Results recorded in this paper show that, with a disease organism transmitted by *S. ornatum*, a susceptible host would have been exposed on average to a 4- to 5-fold increase in the number of potentially infective bites in late evening, and to a far greater number of such bites in early autumn than in mid-season.

Summary.

Females of *Simulium ornatum* Mg. were found to contain abundant fat-body on emergence from the pupa. Unengorged individuals fed on sucrose solution in the laboratory at 16-18°C. and 22-23°C. showed depletion of the fat-body until little remained after 14 days. Females engorged on cattle in the field and kept

in the laboratory at 16–18°C. became gravid in 4–5 days, when virtually no fat-body remained.

Females taken on cattle in the field were divided into two main categories on dissection, (1) those with visible fat-body in the anterior abdominal segments, and of which only 0·06 per cent. contained relict ripe eggs, and (2) those with no fat-body in the corresponding region and of which 16·5 per cent. contained relict ripe eggs, indicating a previous complete gonotrophic cycle. Evidence is given that the two categories approximately corresponded to young flies which had not, and to old flies which had, obtained a blood-meal. There was no evidence that flies rebuilt the fat-body after depletion.

Fresh blood in the mid-gut was encountered more frequently in old flies than in young flies at certain times, suggesting that the former bit more readily or more quickly than the latter.

The average age of the blackfly catches varied inversely with fly abundance except towards autumn, when length of adult life was possibly increased.

The proportion of old flies among the total flies landing on a cow varied during the day and often increased significantly in late evening at the same time as total landing activity increased.

Failure of the proportion of old flies to increase in late evening on two days was possibly caused by the curtailment of fly activity owing to rapid fall of air temperature so that the phase characterised by abundance of old flies was omitted. On another evening the activity of old flies was apparently reduced owing to the effect of wind being greater on them than on young flies.

On days in mid-season, the number of old flies landing on cattle usually remained constant while large fluctuations occurred in the numbers of young flies, except in late evening when the numbers of old and young flies landing tended to change in a parallel manner. This latter state occurred throughout the day in the autumn.

In the case of a disease transmitted by the bite of *S. ornatum*, a susceptible host would have been exposed to a greater number of potentially infective bites in late evening than in the middle of the day in mid-season and to a far greater number of such bites in early autumn than in mid-season.

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AGE-GROUPS AND THE BITING CYCLE IN
ANOPHELES GAMBIAE.
A PRELIMINARY INVESTIGATION.*

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The biting activity of *Anopheles gambiae* Giles has been studied in considerable detail by various workers. Their results are set out in a comparative manner in Table I. The only striking discrepancy shown lies in the relative importance of the mid- and late-night periods. It is apparent from the Table that, with the possible exception of the data given by Hocking & MacInnes (1948), the differences can be partly explained by the type of catch, that is to say whether the mosquitos were caught in the act of biting or whether it was simply their entry into houses that was recorded. The difference suggests that, when the feeding occurs in houses, there is an appreciable pause inside before the host is attacked.

TABLE I.

The percentage of *A. gambiae* entering houses or biting at different periods of the night, as observed by various authors.

Author	Type of catch	1800–2200 hr.	2200–0200 hr.	0200–0600 hr.	Remarks
Kerr (1933)	Biting	5	43	52	Indoors. No catch before 1900
Haddow (1945)	Biting	19	40	41	Outside
Haddow, Gillett & Highton (1947)	Biting	18	31	51	Outside
Mattingly (1949)	Biting	15	33	52	Outside. Mostly <i>A. gambiae</i> var. <i>melas</i> Theo.
Haddow (1942)	Entry	20	44	36	Catches before 1900 and after 0500 hours omitted to avoid entry for resting
Hocking & MacInnes (1948)	Entry	13	>38	<49	No catch before 2030 hr. Last catch continued up to 0630
Holstein (1952)	Entry	20–25	43–49	26–37	Closer comparison not possible. Catches made at odd 2-hour intervals—1900, 2100, etc.
Gillies (see Table II)	Entry	16	48	36	No catch after 0530

The catches have been reduced to three 4-hour periods and approximated as far as possible to local time.

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The present investigation was designed to test the hypothesis advanced by Lumsden (1952) and discussed by Haddow (1954), that certain features of the biting cycle could be explained in terms of the predominant activity of a particular age-group. Such a phenomenon has in fact been demonstrated recently by Davies (1955) in a species of *Simulium*. Recent work on ovarian development in *Anopheles* (Gillies, 1954a) suggested a method whereby this problem could be investigated in *A. gambiae*. It was observed that, under East African coastal conditions, the youngest age-group in the biting population—those feeding for the first time—could be recognised by the failure of the ovaries to develop beyond Christophers' stage II unless a further feed was taken. Such females were described as pre-gravid in contrast to the rest of the population which, after digestion of the blood-meal was complete, was composed of fully gravid females. Some 8 or 9 per cent. of pre-gravids were found to be older females that had already laid eggs at least once, but it was felt that the presence of this small group of exceptional individuals did not seriously detract from the value of the method of age-grouping proposed. After removal of the pre-gravid element, the gravid females could be sub-divided between those with and without sporozoites in the salivary glands. By the use of these groups, the biting population during different periods of the night could be broken up into the following categories:—

- (i) pre-gravid females, nearly all of them being new emergences feeding for the first time,
- (ii) gravid and non-infective females of mixed age but all of them having fed at least once previously, and
- (iii) gravid and infective females, none of them being less than 13 or 14 days old.

The investigation was of a preliminary nature, and further work along similar lines might well be undertaken. This is not possible for me at the present moment, and it was felt that publication of the results achieved might be of interest to other workers in this field.

This work was carried out in Tanganyika, at Tengen, a small village some 26 miles inland from the East African coast at Tanga, in the low-lying foothills of the Eastern Usambara Mountains. The district is moderately densely populated and the land predominantly under cultivation for maize and cassava. The original forest cover has long been cleared and only isolated patches of riverine thicket remain. Cattle are not kept by the people, presumably owing to the infiltration of tsetse flies into the area, and *A. gambiae* has been found to be almost completely anthropophilic (Davidson & Draper, 1953; Gillies, 1954b). Malaria is holoendemic, and the infection rates in this species of mosquito are of the high average level (sporozoite rate 5–10%) often recorded from the humid equatorial zone of Africa.

The work was carried out during the main rainy season from April to early June of 1953. Mean screen temperatures in the district during the period of observation were: maximum 84.8°, minimum 69.1°, daily mean 77°F. The relative humidity in a meteorological screen at 0830 and 1430 hr. averaged 80 and 63 per cent., respectively.

Methods.

Trapping technique.

Two adjacent experimental huts were used, in each of which two paid volunteers slept. The huts were of mud with thatched roofs and low, cotton ceilings. The only means of entry or exit for mosquitos was through a line of shutters, 2½ in. high when open, which were fitted along each of the walls just below the ceiling. When the shutters were closed, the huts were effectively sealed, thus confining the mosquitos that had entered until they were caught by

hand in the morning. The night was divided into three trapping periods, from 1830 (5-17 minutes after sunset)-2200 hr., from 2200-0200 hr., and from 0200-0530 hr. (East African Standard Time). Local time is about 25 minutes behind East African Standard Time. Catches were made in this way for six nights a week for four weeks from 15th April to 10th May 1953, and for three nights a week for the four weeks from 11th May to 7th June. Thus there was a total of 36 nights on which catches were made, spread out evenly over two lunar cycles. On any one night catches could only be made in two periods out of the three, one in each of the two huts available. For this reason, the trapping period in each particular hut was varied from night to night so that, in each cycle of three working nights, one catch was made in each hut in each of the three periods. There were occasional departures from this plan owing to the defection of the sleepers or to some other mishap, and these account for the slight differences in the total number of catches made in each period.

During the day the huts were kept closed. At night, the shutters were opened at the beginning of the period in which trapping was to be done, and closed at the end of it to prevent further entry of mosquitos. The sleepers spent the rest of the night in the hut so that those mosquitos that had already entered, but had not yet fed, could still do so. Supervision of the working of the shutters was always carried out by a responsible officer. This was most important during the night trapping periods, as any carelessness in technique would have invalidated the night's catch in that hut. Closing of the shutters at 0530 was not always supervised, as it was known that, in that particular design of hut, entry at dawn for shelter, rather than for feeding, was on a very small scale, and in any case only recently fed mosquitos were counted and dissected. Mosquitos were collected by hand catching the following morning, unfed or gravid females being discarded.

Technique of age-grouping.

The mosquitos caught each day were transferred to cages and kept till the following day, when they were chloroformed and dissected. The pre-gravids could be recognised by the collapsed condition of the abdomen, and many of them were dissected for examination of the spermatheca. Gland dissections were carried out on the gravid females. Those that had died overnight before the blood-meal was digested were simply counted in with the total numbers of mosquitos and no record was made of their ovarian stage. For each catch, therefore, the following data were recorded:—total catch, numbers pre-gravid

TABLE II.

Biting cycle of *A. gambiae* by age-groups. Geometric means of 22-24 catches.)

	Total no. caught	1830-2200 hr.		2200-0200 hr.		0200-0530 hr.	
		Mean catch	Per cent. of all- night catch	Mean catch	Per cent. of all- night catch	Mean catch	Per cent. of all- night catch
All females ..	3296*	19.1	16.4	55.4	47.8	41.5	35.8
Pre-gravid females	1091	6.3	20.2	13.1	42	11.8	37.8
Gravid females ..	1937	11.6	14.9	39.3	50.5	26.9	34.6

* The discrepancy between the number of "All females" (3,296) and the sum of "Pre-gravid and gravid females" (3,028) is due to the death, overnight, of some individuals, with the result that their ovarian stage could not be determined.

TABLE III.

Pre-gravid rate and fertilisation index of pre-gravid females of *A. gambiae* in different trapping periods.

Week no.	1830 - 2200 hr.				2200 - 0200 hr.				0200 - 0530 hr.			
	No. pre-gravid	Per cent. pre-gravid	Total fed	No. pre-gravid	Per cent. pre-gravid	Total fed	No. pre-gravid	Per cent. pre-gravid	No. pre-gravid	Per cent. pre-gravid	Total fed	No. pre-gravid
1	25	46	54	36	23	158	19	25	19	25	75	
2	5	33	15	13	13	101	14	25	14	25	56	
3	86	63	137	159	43	369	51	35	51	35	146	
4	57	50	113	121	64	189	133	41	133	41	324	
5	5	31	16	28	31	89	42	37	42	37	115	
6	20	28	72	60	32	189	44	25	44	25	174	
7	21	23	91	76	35	218	37	29	37	29	127	
8	18	32	57	12	13	90	17	21	17	21	80	
Total	237	42.7	555	505	36	1403	357	32.5	357	32.5	1097	
Geometric mean of weekly pre-gravid rates (8 weeks)			36.4									29.1
Fertilisation of pre-gravids			No. with sperms	No. with sperms				No. with sperms				No. dissected
			44	117				93				333
Per cent. fertilised				20.4				24.1				27.9

and the percentage of these that had been fertilised, numbers gravid and the sporozoite rate amongst gravid females.

Analysis of the Biting Cycle by Age-groups.

In presenting the results of these catches, Table II compares the biting cycle of the whole mosquito population with that of the youngest age group—the pre-gravids. Table III sets out, in greater detail, data on the percentage of pre-gravids in each trapping period and on the fertilisation rate amongst them. The figures show that entry and feeding of all females was low up to 2200 hours, high in the middle night period, and slightly reduced in the period after 0200 hours. This is in accordance with the findings of previous workers who have studied the entry of *A. gambiae* into houses (see Table I). Of greater interest is the presence of moderate numbers of the youngest age-group—those feeding for the first time—in all periods of the night, showing that the pattern of their feeding activity follows that of the older females fairly closely. On average, a larger proportion of the nightly total of the pre-gravid than of the older females was taken in the early period, and a smaller proportion in the middle period (Table II), and their numbers, as a percentage of the total catch per period, was highest in the early period (Table III). Nevertheless, in four out of the eight weeks' observations, the pre-gravid rate was highest during the middle or late periods (Table III). The fertilisation rate amongst pre-gravids also appears to remain much the same for all periods. The slightly lower fertilisation rate in the early part of the night cannot be regarded as conclusive on such small numbers, nor was it found to be a consistent feature.

In Table IV, the results of gland dissections made on gravid females caught in the different trapping periods are set out. The "all-night" dissections refer to mosquitos caught by spray-catching in the daytime in ordinary African houses in the neighbourhood, or to catches made in the experimental huts on occasions when the entry shutters had been left open all night. The results of dissections from the different trapping periods show that infective females can be caught in all three periods of the night, but it should be noted that the level of infectivity does not appear uniform throughout the night. Too few mosquitos were caught in the first period for the infection rate to be definitely established, but those from the third period are distinctly lower than the all-night sporozoite rate and the difference is greater than three times the standard error.

TABLE IV.

The sporozoite rate amongst females of *A. gambiae* (excluding pre-gravids) trapped during different periods of the night.

	All night	1830— 2200 hr.	2200— 0200 hr.	0200— 0530 hr.
Gland dissections	2365	248	743	606
Positive glands	74	9	20	8
Sporozoite rate	3.1 ± 0.4	3.6 ± 1.2	2.7 ± 0.6	1.3 ± 0.5
Entry rate of gravid females		11.6	39.3	26.9
Entry rate of infective females		0.3 — 0.6	0.8 — 1.3	0.3 — 0.5

Discussion.

These results show quite clearly that the feeding activity of the newly emerged does not differ materially from that of older females. While it is possible that the use of finer time divisions might have revealed short-term variation in age composition of the catches that was obscured by the use of 4-hour periods, these observations do not support the idea that the gross features of the biting cycle in *A. gambiae* are largely dictated by different age-groups coming in to bite as distinct units.

Evidence on the activity of infective females is less definite, but it is noteworthy that they were entering houses in all three main periods of the night and that, all told, about three-quarters of them were caught after 10 p.m. It follows that the risk of malaria infection was similarly distributed.

Summary.

A technique for dividing populations of *Anopheles gambiae* Giles into three age-groups, on the basis of the degree of ovarian development and on the presence of sporozoites in the salivary glands, has previously been described. Using this method, the proportions of the three age-groups amongst females feeding during the three periods into which the night was divided were established for this mosquito in the coastal region of Tanganyika. The feeding activity of newly emerged females followed fairly closely that of older mosquitos, in which entry into experimental huts reached a peak in the period between 2200 and 0200 hr. but remained high from 0200–0530 hr. The younger females were, however, slightly more active in the period before 2200 hr. No difference in fertilisation rate was observed amongst newly emerged females caught in different periods of the night. Infective females were caught in all three periods of the night.

It is not considered that the main features of the biting cycle in *A. gambiae* can be explained in terms of the differential activity of the age-groups recognised in this paper.

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OLFACTORY STIMULATION OF TSETSE FLIES AND BLOWFLIES.*

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A.E.J

Although a considerable volume of work has been published on the response of insects to chemical stimuli, and olfactory receptors have been located on the antennae and palpi, little is known either of the physiological mechanisms concerned in the detection of odours by insects or of the properties of a vapour that are concerned in imparting the odour. This report describes a study of some fundamental factors of olfactory stimulation of tsetse flies, *Glossina* spp., and the blowfly, *Phormia terraenovae* R.-D., made in a laboratory in England. The concentrations of vapours to which these flies respond were determined, and the variation of these concentrations was correlated with that of the physical properties of the compounds used, to provide a guide to the properties of a good olfactory stimulant. The present investigation extends the previous ones of Dethier & Yost (1952) and Dethier (1954b), in which the blowfly, *Phormia regina* (Mg.), served as the test insect, by the use of a greater variety of compounds, by employing blood-sucking flies as test insects, and by a new method of analysis of the results.

Materials and Techniques.

Test insects.

No attempt was made to breed tsetse flies, and the work was dependent on supplies of pupae from Africa. The author is indebted to Dr. K. S. Hocking, Colonial Insecticides Research Organisation, Arusha, Tanganyika, and Dr. T. A. M. Nash, West African Institute for Trypanosomiasis Research, Kaduna, Nigeria, for supplies of *Glossina palpalis* (R.-D.) and to the late Dr. C. H. N. Jackson, East African Tsetse and Trypanosomiasis Research Organisation, Shinyanga, Tanganyika, for supplies of *Glossina morsitans* Westw. On arrival, the pupae were placed in a shallow dish, covered with fine sand and kept at 26°C. and 75 per cent. relative humidity in cages measuring 18" × 18" × 18". The adult flies were kept in the same cages and were given the opportunity to feed daily from a guineapig. Handling in experiments reduced the average life of the adult flies to 2-3 weeks only. Owing to the initial difficulty of obtaining pupae, the variable proportion of flies emerging and the short life of some of them, a continuous supply of tsetse flies was not available and some experiments were performed with the blowfly, *P. terraenovae*, for comparison with the responses of tsetse flies.

Selection of chemicals to be tested.

The compounds tested fell into three groups:—

- (1) Compounds present in decaying animal sweat and excreta, since tsetse flies feed off game. These included the homologous series of liquid mono-carboxylic acids, from formic to pelargonic acid, and a few amines.
- (2) Compounds known to be repellent to other blood-sucking Diptera (Roadhouse, 1953), including a selection of liquid, aliphatic, straight-chain mono-alcohols, ethyl esters and acetates.

* Part of a thesis approved for the degree of Ph.D., University of London.

† Now at National Vegetable Research Station, Wellesbourne, Warwick.

- (3) Hydrocarbons and compounds with more than one functional group, for comparison with the homologous series of acids, alcohols and esters. This group included hydroxy and unsaturated acids and esters, chlorinated esters, and glycols.

Most of these compounds were available commercially and only a few esters had to be prepared specially. The reagents used were freshly distilled or of "Analar" grade.

Apparatus.

The primary response of an insect to either attractive or repellent vapours is movement, but the pattern of behaviour leading to orientation of the insect to the vapour has not been established.

Two sets of apparatus were used in this work. The first measured the activation of flies by known concentrations of a vapour, without giving any information about its attractiveness or repellency. The second apparatus was a "choice chamber", which gave a measure of the attractiveness or repellency of a known concentration of vapour to a population of flies, but without giving any information about the responses of individuals.

Description of the first apparatus.

Air was pumped through towers containing calcium chloride and activated charcoal, to remove water vapour and organic impurities. The dry, odourless air was divided into three streams, the rate of flow of each being regulated by a needle valve and measured on a flowmeter. The first stream was passed straight to a mixing bottle. The second and third air streams were passed through saturators containing the liquid to be tested. The air, saturated with vapour from the liquid, could then be mixed with the first stream in the mixing bottle. By adjusting the rates of flow, the required concentration of stimulant could be obtained. When saturated vapour was required, all three air streams were passed through saturators.

The apparatus was kept in a constant-temperature room maintained at 26°C., the optimum temperature for *G. palpalis* (Nash, 1955). The concentration of saturated vapour was found for each compound used in the work by passing a known volume of air through saturators containing the compound and measuring the resulting loss of weight. The concentration of saturated vapour at 26°C. was also calculated for all the compounds for which adequate data for the variation of vapour pressure with temperature could be found in the literature (Stull, 1947). The maximum variation in the concentration of vapour from the saturators was ± 5 per cent. of the calculated concentration of saturated vapour.

The air containing vapour of known concentration was passed through a five-litre, bolt-head flask containing the test insects. After leaving the flask, the vapour passed into a funnel connected to a extraction fan and so pumped out of the building.

The flask containing the insects was surrounded by a screen, to enable the observer to move freely in the room out of sight of the insects. Observations were made through a small hole in the screen.

The vapour entered the flask through a tube extending to within 2 cm. of the base of the flask and passed out through a short tube at the top. The concentration of vapour in the flask could not immediately attain the incoming concentration and the airflow was studied by filling the flask with a smoke of ammonium chloride and then passing in a clear airstream. The air was found to flow up the sides of the flask, giving a clear band 1-2 cm. thick at the surface, and down the centre, leaving a spherical vortex of smoke which gradually disappeared. Although the distributions of a vapour and a smoke in the flask would not be identical, because of the relatively rapid diffusion of the vapour, the

time taken for the smoke vortex to clear was considered to be equal to that required for the incoming concentration of vapour to be established uniformly throughout the flask. The rate of flow chosen for the experiments was 100 ml./sec. At this rate of flow, vapour at the incoming concentration reached all the flies resting on the inside surface of the flask within 10 sec., and uniform distribution of the incoming concentration of vapour throughout the flask was achieved in 3-4 min.

Preliminary experiments in the first apparatus.

A few preliminary experiments were carried out on the response of tsetse flies to olfactory stimulants, using the vapours from four carboxylic acids present in decaying animal sweat, namely, n-valeric and isovaleric acid and n-butyric and isobutyric acid, and a mosquito repellent, mesityl oxide.

(a) *The response of tsetse flies to isovaleric acid.*—There was a definite pattern of behaviour in the response of both *G. palpalis* and *G. morsitans* to vapour of isovaleric acid. The flies first cleaned their antennae with increasingly vigorous movements of the body and then probed the surface with their mouth-parts. This cleaning and probing continued until the flies began to fly intermittently round the flask. Cleaning and probing reactions were too indefinite to be used as a measure of the response, but the activity of the flies could conveniently be estimated by counting the number of seconds a fly was in the air.

(b) *Variation of response with time.*—Although the average activity of *G. palpalis* was approximately constant over successive five-minute periods of

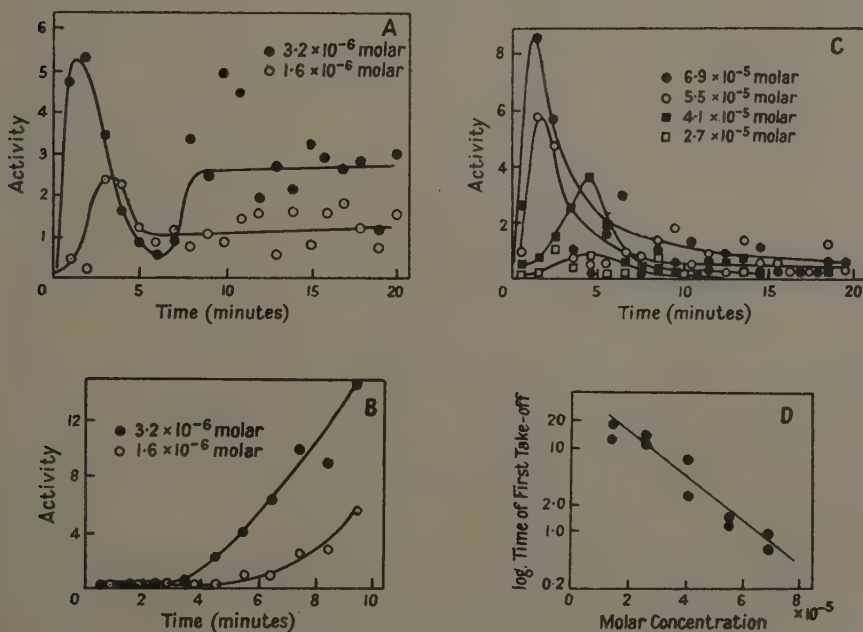


Fig. 1.—Variation of the activity of *G. palpalis* with the period of exposure to vapour of (A) isovaleric acid, (B) aniline and (C) mesityl oxide. Activity is plotted on the vertical axes as the average number of seconds of flight per fly in one minute. (D) variation in the time of first take-off (in minutes) of *G. palpalis* with concentration of vapour of mesityl oxide.

exposure to vapour of isovaleric acid, a more detailed experiment showed that the activity of the flies varied with the period of exposure (fig. 1,A). At the higher concentration used, the activity of the flies reached a peak within the first minute, fell to a lower level and then rose again. At the lower concentration used, the activity of the flies did not rise immediately but reached a peak after 3-4 minutes and then fell to a more or less steady level. There was a similar variation in the response of *G. palpalis* to vapour of mesityl oxide (fig. 1,C), but in this vapour the activity of the flies, after rising to a peak, fell to a level only slightly higher than that in pure air and did not rise again. The peak of activity was lower and occurred later in the lower concentrations of this vapour.

The average time to first take-off of *G. palpalis* varied logarithmically with concentration of vapour of mesityl oxide (fig. 1,D). Observations on the behaviour of the tsetse flies suggested that, with decreasing concentrations of vapour, the increase in the time-lag between the application of the vapour and the first take-off was probably not due to a delay in the perception of the odour by the flies, but to a prolongation of the phases of cleaning and probing at the lower concentrations.

(c) *Olfactory stimulation and toxicity*.—After exposure to the highest concentrations of vapours of isovaleric acid or mesityl oxide, the tsetse flies showed no signs of intoxication. When *G. palpalis* was exposed to similar concentrations of aniline vapour, the flies did not respond to the vapour for five minutes, but then their activity rose until they spun round the flask with uncontrolled movements (fig. 1,B). After only ten minutes in the aniline vapour, a proportion of the flies had received a lethal dose (45% at 3.2×10^{-6} molar* and 20% at 1.6×10^{-6} molar). These differences in the pattern of response may be a distinction between vapours acting as olfactory stimulants and those acting only as toxic fumigants.

(d) *Variation of activity with concentration*.—The activity of *G. morsitans* and *G. palpalis* varied linearly with concentrations of vapours of n-butyric acid and mesityl oxide (fig. 2).

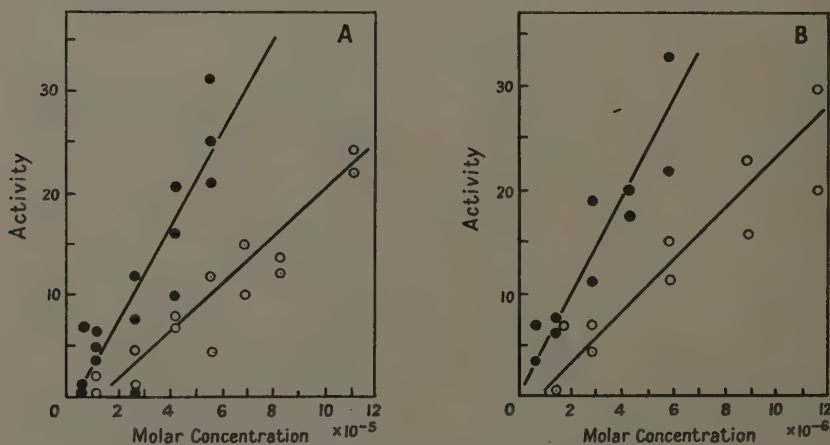


Fig. 2.—Variation of the activity of tsetse flies with concentration of vapour (A) mesityl oxide and (B) n-butyric acid. Closed circles, *G. palpalis*; open circles, *G. morsitans*. Activity is plotted as the average number of seconds of flight per fly in five minutes.

* The molar concentration of a compound is the molecular weight in grammes per litre.

(e) *Activity in relation to the species, condition and sex of the flies.*—*G. palpalis* was twice as active as *G. morsitans* in odourless air and also in the vapours of mesityl oxide and n-butyric acid. The difference between the two species in their response to these odours can therefore be attributed to their initial differences in activity. Similarly, in both species, the males were more active than the females, in either the presence or absence of odours. The state of repletion of the flies did not significantly affect their response to vapours of mesityl oxide or n-valeric acid, although in both vapours the activity of the males was slightly depressed when they were fully engorged.

TABLE I.

Probing responses of tsetse flies.

Test compound	Molar concentration $\times 10^6$	<i>G. morsitans</i>			<i>G. palpalis</i>		
		No. of flies tested	No. of flies probing		No. of flies tested	No. of flies probing	
			in air	in vapour		in air	in vapour
Propionic acid	3.7	12	3	8	12	0	2
	5.9	12	2	7	12	2	12
n-Butyric acid	2.6	12	2	8	8	0	6
	4.5	12	1	5	8	1	7
n-Valeric acid	2.1	12	1	3	12	2	7
	4.2	12	2	9	12	0	9
Butyl acetate	33	12	4	5	8	1	1
	66	12	3	9	8	1	2
Di-n-butylamine	1.0	10	1	1	8	0	0
	2.0	10	0	3	8	0	1
Ethyl dichloracetate	8.5	16	2	6	12	1	1
	17	16	4	13	12	0	0

Observations of probing responses were taken over five-minute exposures.

(f) *Probing responses of tsetse flies.*—Table I gives the proportion of each species of *Glossina* induced to probe by vapours of acids and other compounds, and shows differences in their probing responses. *G. morsitans* probed the surface more frequently than *G. palpalis* in odours and also in odourless air. Both species probed more frequently in the acid vapours, and only these elicited a significant probing response from *G. palpalis*. Since the acids are associated with animal sweat, these results may be relevant to Nash's (1930) observation that covering the antennae of *G. morsitans* with shellac reduced the number that fed on the hosts. However, Dethier (1954a) reported that contact with a heated surface elicited probing responses from tsetse flies, and that olfactory stimulation did not. Reduction of the probing response to heat followed extirpation of the antennae, suggesting that Nash's results could be due to interference with the thermal receptors rather than the olfactory receptors.

(g) *The effects of external factors on the response to odours.*—The activity of *G. morsitans* in odourless air and in vapour of isobutyric acid was not affected by variations in the light intensity (from 25 to 250 ft.-candles), relative humidity (from 0 to 70 per cent.), or population density (from one to five flies in the flask).

The response to vapour of isovaleric acid or mesityl oxide varied with the rate of flow. This might reflect the time taken for the concentration of vapour in the flask to reach the incoming concentration, and there was no evidence of any direct effect of airflow on fly behaviour. The results demonstrate the lack of response of tsetse flies to external stimuli under the experimental conditions. Visual stimulus was an exception, since the flies were readily disturbed by movements near the flask. No experiments on the effects of temperature on the activity of the flies were carried out.

(h) *Consistency of the results.*—The preliminary experiments indicated that comparable results for the activation of *G. morsitans* could be obtained over a period of several months. For example, the results for the activation of *G. morsitans* by vapour of mesityl oxide shown in fig. 2 were obtained three months after the results given in fig. 3. If flies of the same age and state of repletion could have been used, more consistent results might have been obtained. Bias in the results of any one experiment was eliminated by careful randomisation of the flies, as described below. Bias due to variation in the condition of the flies used in the different experiments was checked by counting the number of seconds each fly was in flight in five minutes in pure air. This activity showed only slight variability. Samples of *G. palpalis* were obtained from two sources and travelled under varying conditions, so that the results from different batches were not sufficiently constant for this species to be used for comparison of olfactory stimulants.

The choice-chamber olfactometer.

Olfactometers of the type designed by Hoskins & Craig (1934) to measure the olfactory responses of house-flies have been used successfully with other active insects, such as mosquitos (Willis & Roth, 1952) and blowflies (Dethier, 1954b). The apparatus used in the present work was similar to the original design and will not be described in detail.

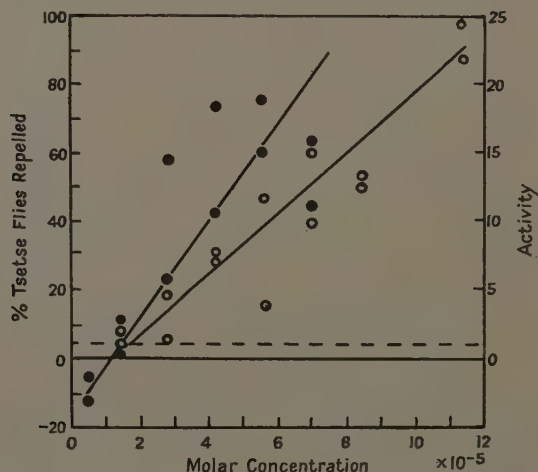


Fig. 3.—Stimulation of *G. morsitans* by vapour of mesityl oxide. Closed circles: percentage flies repelled in the choice chamber. Open circles: activity of the flies in the flask (the average number of seconds of flight per fly in five minutes). The broken line represents the average level of activity of the flies in pure air.

A measure of the attractiveness or repellency of a known concentration of vapour was given by the distribution of the test insects on two adjacent port-holes in the side of a cage. In the absence of vapour, there was an equal number of insects on each port-hole and the redistribution of the insects when an odorous substance was passed through one port-hole depended on their making a continuous choice between the port-holes.

In this work, satisfactory results could be obtained with the blowflies (*P. terraenovae*), which are active insects, but not with the tsetse flies which were more lethargic. Although inconsistent and variable results were frequently obtained with the latter, concentrations of mesityl-oxide vapour that activated *G. morsitans* in the first apparatus were repellent to this species in the choice chamber (fig. 3). The possibility that mesityl oxide was attractive at lower concentrations was not investigated, but 1.5×10^{-6} molar isovaleric acid, which raised the activity of examples of *G. palpalis* to four times its normal value, was attractive to this species in the choice chamber. There was no observable difference in the response of tsetse flies to attractants and repellents in the first apparatus, and the range of concentrations used may have covered both attractive and repellent concentrations of some compounds. Either method thus gave comparable results, although the first could not show whether a vapour was attractive or repellent. However, the first apparatus had considerable advantages over the choice chamber. Results could be obtained easily and quickly using small samples of flies, whereas the choice chamber required large numbers of flies and results could only be compiled slowly. The choice chamber was therefore discarded, after a few preliminary experiments had led to this conclusion.

Standard procedure used in the measurement of activity.

As a result of the experience gained from the preliminary experiments, the following standard procedure was adopted in the main experiments, using the first apparatus, described above.

About midday, individuals of *G. morsitans* were allowed to feed from a guinea-pig for an hour, after which about one-third of them would be fully engorged. The flies were then caught in specimen tubes which were placed in sequence in a rack. Next morning the flies were sexed and any abnormal ones rejected. Five minutes before an experiment, five flies of the same sex were selected at random from the racks. Samples of male and female flies were used alternately. The flask was connected into the apparatus and dry, odourless air passed through at 100 ml./sec. for two minutes, a sufficient period for the flies to resume normal activity. A count was then taken of the number of seconds for which each fly was in flight during five consecutive minutes; this total was taken as the measure of activity. Saturated vapour of the chemical to be tested was then introduced into the air stream, to give a known concentration. After allowing 30 sec. for the concentration at the inner surface of the flask to reach that of the incoming airstream, a count was taken of the number of seconds for which each fly was in flight during each minute for five minutes, and the time at which each of the five flies first left the surface was also recorded. The experiments were carried out at 26°C. and in light intensity of 25 ft.-candles. After each sample of flies had been tested, odourless air was passed through the apparatus for 5-10 minutes, to remove the vapour before introducing the next sample.

Results of the Main Experiments.

Experiments with tsetse flies.

(a) *Determination of the standard molar concentrations.*—The activity of *G. morsitans* in known concentrations of vapours of the compounds was measured, using the standard procedure described above. The flies did not respond to concentrations of vapour below a threshold value characterising each compound.

In concentrations about ten times greater, the flies flew round the flask with slightly uncontrolled movements. These values were therefore selected for the lower and upper limits of the range of concentration tested and the duration of flight of two samples, each of five flies, was recorded in five to eight concentrations in this range.

In fig. 4, the activity of *G. morsitans* is plotted against the concentration of vapour of homologous acids and alcohols, expressed as percentage saturation of vapour in air. Similar results were obtained with other compounds. Over the range of concentration that stimulated the flies, their activity varied approximately linearly with concentration and the best-fit regression line of activity on

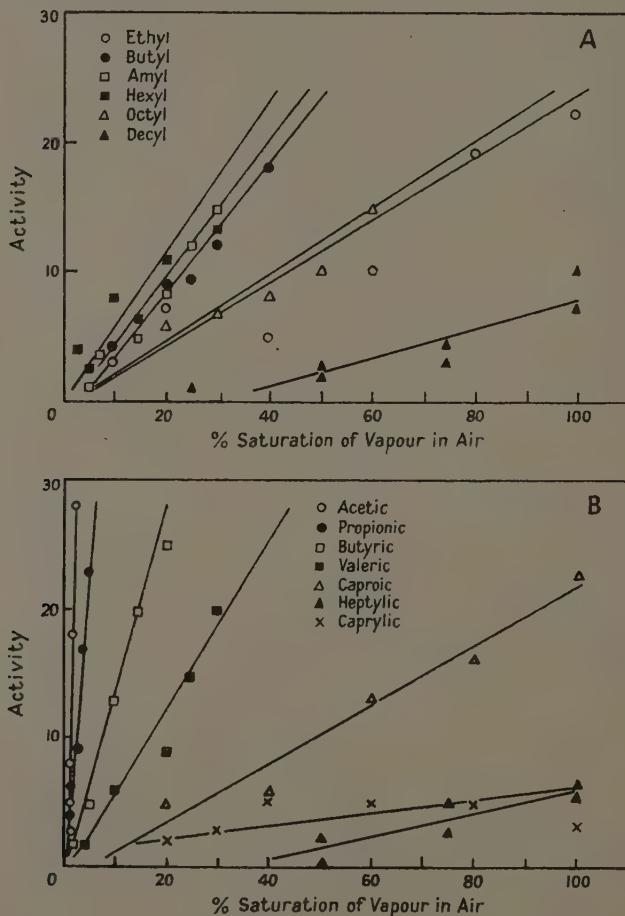


Fig. 4.—Variation of the activity of *G. morsitans* with concentration of vapour of (A) homologous alcohols and (B) homologous acids. Each point gives the average number of seconds of flight per fly in five minutes for one sample of five male and one sample of five female flies.

concentration was determined for each compound. From this regression line was determined the percentage saturation of vapour required to raise the activity of the flies to a standard level of ten times that in pure air (about one second of flight per fly in five minutes in the case of *G. morsitans*). The product, percentage saturation \times molar concentration of saturated vapour, gave the molar concentration of vapour required to raise the activity of the tsetse flies to the standard level. This was termed the standard molar concentration, and the values of this for all the compounds tested are given in Table II. The 5 per cent. confidence limits were calculated and found to be not more than ± 20 per cent. of the standard molar concentration.

(b) *Determination of threshold concentrations.*—For each compound, the maximum concentration of vapour that would not raise the activity of *G. morsitans* was calculated by inserting the value of the activity in odourless air (i.e., about one second of flight per fly in five minutes) into the regression equation. Concentrations of n-hexanol, n-butyric acid, butyl acetate and ethyl valerate descending to one hundredth of such calculated values were tested and failed to elicit any response from the flies, showing that the calculated concentrations were in fact threshold concentrations. These thresholds were thresholds of response, but not necessarily of perception; lower concentrations may be detected by the flies without eliciting a visible response. The error in estimating the threshold concentrations is high, and only values greater than the 5 per cent. confidence limits are given in Table II.

Experiments with blowflies.

The pattern of response of *P. terraenovae* to olfactory stimulants was similar to that of tsetse flies. The blowflies first cleaned their antennae; they then walked round the flask until finally they were stimulated to fly. Their activity was measured for a number of acids, alcohols and esters, using the standard procedure.

The average level of activity of *P. terraenovae* in odourless air was three seconds of flight per fly in five minutes, compared with one second for *G.*

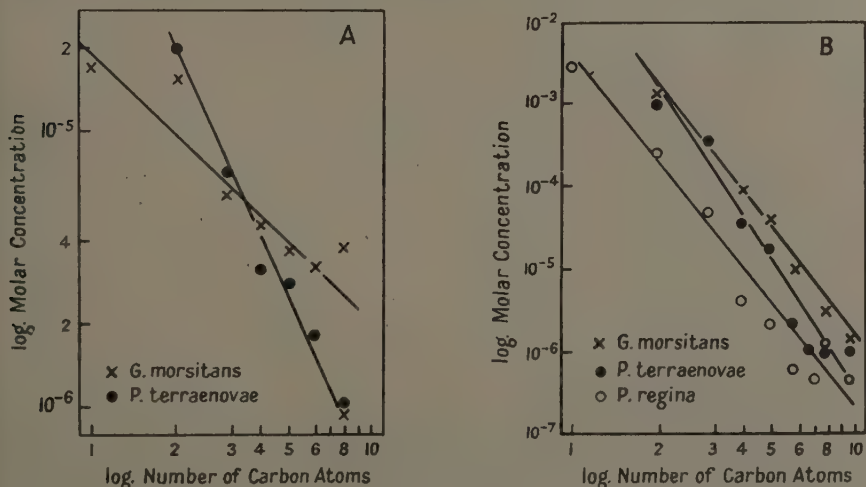


Fig. 5.—Variation of standard molar concentrations with number of carbon atoms: (A) homologous acids, (B) homologous alcohols.

TABLE II.

Values of selected physical constants of compounds tested, and of concentrations required to induce standard fly activities.

Y_1	=	Log standard molar concentration
Y_2	=	Log threshold molar concentration
Y_3	=	Log saturated vapour pressure at 26°C. in mm. Hg, measured by the author or taken from Stull (1947)
X_1	=	Log water solubility in per cent. by weight of solute in solution. Anon. (1941) or Seidell (1941), or calculated from equations by von Erichsen (1952)
X_2	=	Dipole moment in debye units (Wesson, 1949)
X_3	=	Log dielectric constant (Maryott & Smith, 1951, or Phadke, 1952)
X_4	=	Apparent molecular volume in cc. $\times 10^{23}$, calculated from molecular weight and density (Anon., 1933)
X_5	=	

Compounds	<i>G. morsitans</i>		<i>P. terraenovae</i>		X_1	X_3	X_4	X_5	
	Y_1	Y_2	Y_1	Y_2					
Group I—Acids									
Formic acid	..	Toxic	-4.70	..	1.23	2.00	1.73	0.82	9.5
Acetic acid	..	-5.24	-5.14	..	0.61	2.00	1.74	0.52	12.4
Propionic acid	..	-5.70	-5.48	-6.49	0.02	0.75	1.74	0.49	15.3
Butyric acid	..	-5.80	-5.55	-6.38	-0.42	0.59	1.74	0.42	18.3
Valeric acid	..	-5.96	-5.74	-6.70	-0.85	0.03	1.74	0.43	19.0
Caproic acid	..	-6.15	-1.26	-0.62	1.74	0.41	23.4
Heptylic acid	..	-6.33	-6.04	-7.07	-1.74	-1.14	1.74	0.41	26.3
Caprylic acid	..	-6.48
Pelargonic acid	..	> sat. vap.
Acrylic acid	..	-5.24	-5.82
Lactic acid	..	> sat. vap.	-4.64
Group II—Alcohols and esters									
Ethanol	..	-3.55	-3.00	-4.01	1.78	2.00	1.66	1.39	9.6
Isopropanol	-3.40	-4.36	1.68	2.00	1.69	1.30	12.6
n-Propanol	-3.52	-4.36	1.33	2.00	1.69	1.30	11.8
Isobutanol	-4.52	..	1.04	0.90	1.69	1.23	15.2
n-Butanol	..	-4.85	-4.41	..	0.85	0.88	1.69	1.23	15.2
n-Pentanol	..	-5.01	-4.80	..	0.51	0.34	1.69	1.14	17.9
n-Hexanol	..	-5.57	-5.70	..	0.08	-0.24	1.69	1.12	20.7
sec-Hexanol	..	-4.59
tert-Hexanol	..	-4.68
Cyclohexanol	-5.27	-6.16	0.18	0.56	1.69	1.18	16.9
n-Heptanol	-5.96	-6.72	-0.55	-0.10	1.69	1.06	23.5
n-Octanol	..	-6.08	-5.85	-6.43	-0.77	-1.23	1.69	1.01	26.1
n-Decanol	..	-6.30	-5.96	-6.59	-1.62	-2.22	1.69	0.91	31.7
Benzyl alcohol	..	-5.92
Ethylene glycol	..	> sat. vap.	> sat. vap.	> sat. vap.

TABLE II—continued

Compounds	<i>G. morsitans</i>		<i>P. terraenovae</i>		X_1	X_2	X_3	X_4	X_5
	Y_1	Y_2	Y_1	Y_2					
Ethyl acetate	1.98	0.91	1.86	0.78	16.3
Ethyl butyrate	-4.22	..	1.26	-0.21	1.89	0.71	20.0
Ethyl valerate	-4.57	..	0.76	-0.52	1.89	0.67	24.7
Ethyl caproate	0.23	-1.20	1.89	0.61	27.4
Ethyl caprylate	0.48	-2.14	1.89	0.56	32.6
Ethyl caprate	-1.02	2.82	1.89	0.54	38.5
Ethyl laurate	-1.42	-4.00	1.89	0.54	43.7
Ethyl myristate	>sat. vap.	>sat. vap.	1.08	0.36	1.85	0.70	19.8
Butyl acetate	-4.05	-4.60	0.86	-0.80	1.91	0.68	24.6
Amyl acetate	-4.43	-5.06	-5.22	..	0.26	-1.35	1.91	0.61	26.9
Hexyl acetate	-4.96	-5.57	0.23	-1.35	1.90	0.60	24.4
Cyclohexyl acetate	-5.57	-6.23	-0.62	-2.58	1.91	0.56	32.3
Octyl acetate	-5.43	-6.08	-1.12	-3.74	1.91	0.54	37.8
Decyl acetate	-5.85	-6.30	-2.36	-5.00	1.91	0.54	44.0
Lauryl acetate	-6.52	-6.82					
Cetyl acetate	>sat. vap.	-7.25					
Group III—Miscellaneous Compounds									
Ethyl crotonate	-4.37	-4.77	0.74	0.23	2.64	1.06	18.2
Ethyl lactate	-4.92	-5.62	0.49	-0.52	2.61	1.01	20.2
Ethyl monochloracetate	-4.52	-5.46	0.17	-1.32	2.55	0.89	24.4
Ethyl dichloracetate	-4.54	-5.11	-0.38	2.00	2.33	1.11	16.5
Ethyl trichloracetate	-4.49	-5.18					
Ethylene glycol monoacetate	-4.74	-5.09					
Dimethyl phthalate	>sat. vap.	-6.70					
Pyridine	..	Toxic					
Ethylene diamine	..	Toxic					
Di-n-butylamine	-5.72	-6.42					
β -Phenyl ethylamine	-5.82	-6.42					
Acetone	-2.89	-3.77					
Hexane	..	Toxic					
Decane	..	Toxic					
Benzene	..	Toxic					
Toluene	..	Toxic					

morsitans. The standard level of activity was accordingly chosen as thirty seconds of flight per fly in five minutes instead of the ten seconds selected for *G. morsitans*. The activity of *P. terraenovae* varied linearly with concentration of vapour and the standard molar concentration for each compound was calculated from the regression line of activity on concentration.

Comparison of results with tsetse and blowflies.

In fig. 5,A, the logarithms of the standard molar concentrations for the carboxylic acids are plotted against the logarithm of the number of carbon atoms in the acid. There is a marked similarity between the results for *G. morsitans* and *P. terraenovae*. Fig. 5,B shows, similarly, the relationship between the standard molar concentrations of alcohols and the number of carbon atoms. Also plotted on this graph are the results given by Dethier & Yost (1952) for the molar concentrations of alcohol vapours required to repel 50 per cent. of examples of *P. regina* from one port-hole of an olfactometer of the type designed by Hoskins & Craig. There is a significant difference in the position of the lines, but not in their slope. The repulsion of 50 per cent. of the flies is probably a less stringent criterion of response than their activation to the standard level, and the concentrations required probably approximate to the threshold rather than the standard concentrations for *P. regina*. In view of the fact that three species of flies and two methods of estimating their responses to odours are concerned, the results are remarkably similar.

Analysis of the Results.

Previous workers have correlated the biological activity of narcotics and poisons with the physical properties of their molecules. Ferguson (1939), in a review of the literature, showed, for a number of substances, that the concentrations required to induce narcosis or death in several organisms varies with vapour pressure, if the substance is applied in the vapour phase, or solubility, if it is applied in solution. Thus he found that the thermodynamic activities at which substances are equally effective as narcotics or poisons are nearly constant. There are, however, deviations from this rule, both within and between homologous series, and he attributed these to chemical as distinct from physical action. On the other hand, another physical property, or combination of physical properties, might account more satisfactorily for the variation, in different chemicals, in the concentrations that have equal biological activity. Indeed, Mullins (1954) shows that the concentrations of substances required to induce narcosis are more likely to be determined by a combination of thermodynamic activity and molecular volume than by the former alone.

The standard and threshold concentrations decrease as each homologous series is ascended, in a similar manner to iso-narcotic concentrations of vapour (Ferguson, 1939). The thermodynamic activity of the vapour is the ratio of the vapour pressure to the saturated vapour pressure, expressed as a fraction or a percentage. Fig. 4, in which the activity of the flies is plotted against the percentage saturation of the vapour, shows that the compounds are not equally effective at equal thermodynamic activities, but that there is a regular variation in each series.

The physical properties of homologous compounds vary regularly in a series, and plotting the standard molar concentration against any single physical factor will give an apparent relationship between the two. It was therefore necessary to use a special statistical technique to determine the relative importance of different properties. This consisted of performing a multiple regression analysis, which eliminated any intercorrelations between the physical properties before testing the dependence of the standard molar concentration on each of them.

In deciding what physical properties to include in the analysis, account was

taken of the following considerations. The olfactory sensilla, situated on the antennae and palpi of Diptera, are thin-walled receptors associated with primary sense cells. To be effective, an olfactory stimulant must cause an excitation of the nerves leading from the sensilla. The vapour molecules must therefore be adsorbed on the surface of the sensilla, penetrate the cuticular membrane, reach the site of action in the sense cells and initiate a nerve current. Any of these processes could be the limiting factor in the fly's detection of olfactory stimulants and thus determine the concentration of vapour required. The physical properties that might be expected to affect the processes listed above are saturated vapour pressure, molecular volume, oil solubility, water solubility, oil-water partition coefficient and (since nerve currents are essentially electrical phenomena, initiated by the alteration of a membrane potential) the electrical properties of the molecule, namely, dipole moment and dielectric constant. Nearly all the compounds were miscible with oil, so that oil solubility could be excluded from the calculations, and oil-water partition coefficients could not be included because there are insufficient data on them in the literature. This left five physical properties to be used as independent variables in the partial regression analysis.

In his study of the repellency to the blowfly, *P. regina*, of the vapours of homologous alcohols and aldehydes, Dethier (1954b) found that within each homologous series the concentration of vapour required to repel half the blowflies generally conformed to Ferguson's theory of narcotic action. However, there were discrepancies among the higher and lower members of the series, and also between the series, which suggested that thermodynamic activity alone is not a sufficient basis for comparison of repellents. A partial regression analysis, in which Dethier's values of the concentrations of vapours repellent to *P. regina* were related to vapour pressure, molecular volume, water solubility, dipole moment and dielectric constant, was accordingly carried out in the same way as the analysis relating the standard molar concentrations for the two other test insects to the same five physical properties.

The variation in the values, for different compounds, of the standard molar concentrations (C) necessitated the use of the logarithms of these quantities in the calculation. In a homologous series of compounds, $\log C$ varied approximately linearly with the number of carbon atoms, and to determine whether logarithms of the independent variables should be used, the values for each property were plotted against the number of carbon atoms, for each series. Vapour pressure, water solubility and dielectric constant varied approximately logarithmically with the number of carbon atoms, and dipole moment and molecular volume varied linearly. Accordingly, logarithmic transformations were used for the first three properties, but not for the last two. The values of the five physical properties used in the calculation are given in Table II.

The partial regression analysis is based on the assumption that the logarithm of the standard molar concentration can be related to the five physical properties by a linear regression equation of the form

$$\log C = b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + c$$

where $x_1 = \log$ vapour pressure

$x_2 = \log$ water solubility

$x_3 = \text{dipole moment}$

$x_4 = \log$ dielectric constant

$x_5 = \text{apparent molecular volume}$

$b_1 \dots b_5 = \text{the corresponding partial regression coefficients}$

$c = \text{a constant}$

The method of calculating the partial regression coefficients and the corresponding variance ratios is a well-established procedure and is described in detail by Goulden (1952).

and the percentage of the variability not attributable to the physical properties can be accounted for by the error in estimating the concentrations. However, in the case of *P. terraenovae*, 18 per cent. of the variability is not attributable to the physical properties, although there is no greater error of estimation of the standard molar concentrations.

The calculation of the partial regression coefficients for *P. terraenovae* illustrates well the disadvantage of this type of procedure. The compounds tested on this species were not well chosen for the purposes of the calculation, because a sufficiently wide range of the physical properties was not covered. There is only a very small variation in the dipole moments of the compounds concerned, and this imposes restrictions on the relative values of the other properties. In general, the values of the partial regression coefficients will change as more compounds, covering a wider range of physical properties, are included in a calculation; if any factor genuinely determines the standard molar concentrations it should then become more significant. Conversely, any spurious correlations of the standard molar concentrations with a physical property, found when testing a small number of compounds, will become insignificant when a greater number and variety of compounds are tested. It is particularly satisfactory that in the present investigations the significance of dielectric constant and vapour pressure increases as the number of compounds increases. Thus, when a restricted range of compounds was tested on *P. terraenovae*, only dielectric constant approached significance. Dethier (1954b) tested the same number of compounds on *P. regina*, but covered a wider range of dipole moments. In this case, dielectric constant was significant. In the case of *G. morsitans*, a large number of more diverse compounds was tested, and both dielectric constant and vapour pressure were shown to be highly significant.

Vapour pressure and dielectric constant both attain significance in the cases of both the blowflies when the other three factors are dropped from the analysis. In the five-factor analysis, the non-significance of vapour pressure may be due to the high correlation between this property and the other four, and is again a consequence of the restricted range of compounds tested. A number of compounds tested on *G. morsitans* could not be included in the calculation since complete data on their physical properties are not given in the literature. For all these compounds, the standard molar concentrations approached the values expected from consideration of vapour pressure and dielectric constant, except where the compounds had very high or very low dipole moments. High dipole moment lowered, and low dipole moment raised, the standard molar concentration; this fact is consistent with the negative partial regression coefficient found in the multiple regression analysis in the case of this property and this insect.

Vapour pressure also has a secondary effect on the olfactory stimulation of insects. The standard and threshold concentrations of vapour may be determined by the vapour pressure and dielectric constant of a compound, but the vapour pressure must be high enough to maintain this concentration. If it is too low to do so, as with some of the higher members of a homologous series, the compound will have no effect on the insects.

Discussion.

Physiological action.

This examination of a fairly wide selection of compounds leads to the conclusion that a good olfactory stimulant should be a liquid of low vapour pressure, low dielectric constant, and, though of less importance, high dipole moment. The following considerations suggest that the vapour pressure may determine the availability of the olfactory stimulant to the sensilla, while the dielectric constant may determine the concentration of stimulant required at the site of action to stimulate the nerves leading from the sensilla.

Under equilibrium conditions, the concentration of a narcotic at the site of action in the cells of an organism is determined by the thermodynamic activity of the narcotic in the external medium (Ferguson, 1939). If the narcotic is applied in the vapour phase, the thermodynamic activity is the ratio of the vapour pressure to the saturated vapour pressure and therefore the external concentration will depend on the vapour pressure. This explanation of the correlation of iso-narcotic concentrations with vapour pressure is equally applicable to the correlation of the standard molar concentrations of olfactory stimulants with vapour pressure. In view of the small dimensions of the sensilla, it is reasonable to assume that the penetration of the gas molecules into the sensilla is very rapid and that equilibrium between the phases of the cells is quickly attained. The thermodynamic activity of the compound at the site of action will then be equal to its thermodynamic activity in the external vapour phase and the concentration of vapour required in the external phase will be dependent on the saturated vapour pressure.

Propagation of a nerve action current appears to involve the local depolarisation of a nerve membrane (Hodgkin, 1951), which alters the permeability of the membrane to sodium ions; these enter the fibre at high rate, reversing the potential across the membrane and providing the current for depolarising adjacent regions of the nerve membrane. Alternation of depolarisation and the consequent sodium influx propagates the nerve impulse. The molecules of an olfactory stimulant may excite the olfactory nerves leading from the sensilla by producing a local depolarisation of membranes in the sensilla which may be continuous with the nerve membranes. Depolarisation could be affected by the acceleration or retardation of enzyme systems producing ionic changes at the membrane (*cf.* Baradi & Bourne, 1951) or by adsorption of the olfactory stimulant on the membrane. The first mechanism would be unlikely to be affected by the dielectric constant of an olfactory stimulant, although some compounds, of a different type from those tested in this work, such as mercaptans, may affect enzyme reactions and stimulate the sensilla by this mechanism.

Nerve membranes consist basically of a lipid layer between two layers of orientated protein molecules (Schmitt & Bear, 1939). Across the membrane there is a measurable potential difference, due largely to differences in the mobilities of ions in the membrane, leading to differences in the concentrations of ions across the membrane, but probably including also a potential difference at the surface of the membrane, caused by the formation of electrical double layers on the orientated protein molecules. Solution of lipophilic molecules in the lipids of the membrane would not be expected to alter the membrane potential unless the molecules were polar and orientated so that the dipoles lay in the same direction. The compounds tested in this work consisted of molecules with polar groups attached to lipophilic carbon chains of varying length. The polar groups would tend to orientate the molecules in the membrane potential, probably at the surface of the protein layer, and the carbon chain would tend to orientate molecules in the lipid layer. Since the dielectric constant is a measure of the orientation of molecules in an electric field, it will also be a measure of the ability of the lipophilic chain to orientate the dipoles against the membrane potential. With a decrease of dielectric constant, the molecules should thus become more uniformly orientated and fewer molecules would be required to produce a threshold change in potential.

If the orientation of polar molecules provides a mechanism of local depolarisation of the membranes in the sensilla, the magnitude of the dipole of the molecules would be expected to affect the number of orientated molecules required to produce a threshold change in potential. However, as dipole moment was not shown to be a highly significant factor in determining the standard molar

concentrations, it may be concluded that the orientation of the dipoles in the membranes is more important than their magnitude.

Thus, the dielectric constant of an olfactory stimulant may determine the concentration of the compound required in the membranes to induce depolarisation, and the vapour pressure may determine the external concentration of vapour required to produce this concentration in the membranes.

Practical applications of the results.

An olfactory stimulant might be used to increase the efficiency of insecticides sprayed over bush infested with tsetse flies. A volatile stimulant included in the insecticidal solution would evaporate from the spray droplets. The vapour might penetrate to flies resting outside the air currents carrying the spray and stimulate the flies to move into the spray, thus increasing the number of flies which would receive a lethal dose of insecticides. For this purpose it is not necessary to know whether the olfactory stimulant is attractive or repellent.

Work by Yeo & Thompson (1954) on the evaporation of kerosene from spray droplets suggests, indirectly, that any oil-soluble compound boiling in the range 150–250°C. would be suitable. Di-n-butylamine, β -phenyl ethyl amine, caproic, heptylic and caprylic acids, octyl and decyl alcohols, ethyl caprate and octyl and decyl acetates stimulated *G. morsitans* at concentrations of 10^{-4} to 10^{-5} g./litre, and further work on these compounds in the field might be profitable.

Compounds boiling above 250°C. which activated *G. morsitans* at 10^{-4} to 10^{-5} g./litre were lauryl and cetyl acetates, ethyl laurate and dimethyl phthalate. However, the concentrations of these substances which activated tsetse flies were near the saturated concentrations and it is unlikely that saturated vapour of any substance could be produced by evaporation from spray droplets, except in a confined space.

On the other hand, the low volatility of these compounds suggests that these substances might be good protective repellents. Dimethyl phthalate has been found to give some protection against tsetse flies (Findlay, Hardwicke & Phelps, 1946) and cetyl acetate should give protection over longer periods, since it activates *G. morsitans* at lower concentrations and has a lower vapour pressure than dimethyl phthalate.

Attractive scents are known to increase the number of tsetse flies caught in traps (Chorley, 1948). The addition of some of the carboxylic acids to traps might increase the catch, since these compounds induce probing and are probably attractive to tsetse flies.

Summary.

An apparatus for exposing insects to known concentrations of vapours from odorous liquids is described; it was used in a laboratory in England to test adult tsetse flies, *Glossina palpalis* (R.-D.) and *G. morsitans* Westw., obtained from Africa as pupae, and the blowfly, *Phormia terraenovae* R.-D. There is a definite pattern of behaviour of the flies in olfactory stimulants, in the final phase of which they are excited to fly. The number of seconds for which a fly is in flight gives a measure of its activation by the vapour. The method gives results comparable with those obtained with a standard choice-chamber technique and has considerable practical advantages for work with tsetse flies. The effects of concentrations of vapour, period of exposure, condition of the flies and external conditions on the response of tsetse flies to vapours were studied.

The response of *G. morsitans* and *P. terraenovae* to a selection of aliphatic acids, alcohols, esters and amines was determined, using a standardised procedure. There is a marked similarity between the two species in the concentrations of vapours required to activate them. The standard molar concentration,

defined as the concentration of vapour required to raise the activity of the flies to a standard level of ten times that in pure air, was calculated for each compound tested from the regression of activity on concentration. Threshold concentrations (the highest concentrations that failed to raise fly-activity) were also calculated from the regression equations.

A multiple regression analysis was used to relate the standard molar concentrations to five physical properties of the compounds, namely vapour pressure, water solubility, molecular volume, dipole moment and dielectric constant. A similar analysis was carried out relating median repellent concentrations (those repelling 50 per cent. of the flies in a standard choice chamber) to the same five physical properties, using data previously obtained by Dethier (1954b) for the blowfly, *Phormia regina* (Mg.). The results suggest that a compound having low vapour pressure, low dielectric constant and, possibly, high dipole moment, is likely to be a good olfactory stimulant. A mechanism is suggested for olfactory stimulation of flies, depending upon the orientation of polar molecules in the membranes of the olfactory sensilla.

Possible practical applications of olfactory stimulants for tsetse flies are discussed.

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OLFACTORY STIMULATION OF *GLOSSINA PALPALIS* (R.-D.) BY COMBUSTION PRODUCTS FROM PETROL ENGINES.

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A brief attempt has been made to follow up the observation by Napier Bax (1937) that exhaust fumes from old motor cars and lorries stimulate the tsetse fly, *Glossina swynnertoni* Aust., in a similar manner to odours from oxen. The observation suggested that very low concentrations of a compound present in combustion products from petrol engines might stimulate tsetse flies. In order to determine whether any such compound was present, the effects of exhaust gases and vapour from sump oil on the activity of *Glossina palpalis* (R.-D.) was measured, using a technique described in the preceding paper (Hughes, 1957).

Experiments and Results.

A sample of sump oil was obtained from a garage; it was collected from motor cars of all ages and would be expected to contain most of the compounds normally found in the sumps of cars. The crude oil, an evil-smelling, black, viscous liquid, was distilled and the fraction boiling below 230°C. was collected. The residue, which had an unpleasant odour not identical with that from the original oil, was distilled under vacuum to extract liquids with higher boiling points. The yield from two litres of crude oil was approximately 90 ml. of ordinary distillate and 20 ml. of vacuum distillate. The ordinary distillate consisted of an aqueous acidic layer and a neutral upper layer, which were carefully separated.

Saturated vapour from the aqueous layer was tested on *G. palpalis* by the standard procedure for measuring fly activity (Hughes, 1957) and was found to be without effect. The upper, hydrocarbon layer was distilled through an eight-inch fractionating column, and seven fractions, distilling over successive temperature ranges of about 15°C., were collected. The vacuum distillate was separated, by distillation under reduced pressure (12.5 mm. Hg) through a short column, into two fractions having boiling ranges of 110–170°C. and 170–250°C., respectively.

Five to seven concentrations of vapour from each fraction were tested on *G. palpalis*, using the standard procedure. The activity of the flies varied linearly with concentration of vapour. The threshold concentration of vapour (the maximum that did not raise fly activity above that in pure air) and the standard concentration (that required to raise fly activity to ten seconds of flight per fly during five minutes) were calculated for each fraction (Table I). The threshold and the standard concentrations of vapour decreased as the saturated vapour pressure of the fraction decreased. In this respect the fractions behaved as would be expected of a series of homologous or similar compounds of increasing boiling point (Dethier, 1954), and the results provided no evidence for the existence of an exceptionally active stimulant in any one fraction.

Exhaust gas from an old motor car was tested on *G. palpalis*. A 100-litre flagon was filled with exhaust gases, which were then forced through a flask containing flies by running water into the flagon. The exhaust gases did not raise the activity of the flies above the level found in pure air. It was possible,

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TABLE I.
Standard and threshold concentrations of vapour from distillates of motor-car sump oil,
in relation to activity of *G. palpalis*.

Fraction	Boiling range (°C.)	Yield (g.)	Average concentration of saturated vapour (g./litre)	Concentration (mg./litre)	
				Standard	Threshold
<i>Ordinary distillates</i>					
I	85-100	5			Toxic
II	100-120	10	0.15	9.5	3.5
III	120-135	8	0.067	4.7	1.7
IV	135-145	8	0.044	3.7	1.1
V	145-163	14	0.026	2.2	0.61
VI	163-178	8	0.018	1.6	0.98
VII	178-225	12	0.0044	1.3	0.10
Residue	225	< 1			
<i>Vacuum (12.5 mm. Hg) distillates</i>					
VIII	110-170	10	0.0020	0.94	0.10
IX	170-250	10	0.0004		No effect
Residue		< 3			

Standard concentration of vapour calculated as that required to raise fly activity to 10 sec. total flight per fly during 5 minutes.

Threshold concentration calculated as the maximum that did not raise fly activity above that in pure air.

however, that the odorous substance was adsorbed on the finely divided smoke particles of the exhaust, which may have been deposited on the sides of the flagon, and that the smell was thus removed.

Discussion.

This superficial examination of the effect on *G. palpalis* of distillates of sump oil, and exhaust gases, was sufficient to show that compounds that stimulate tsetse at very low concentrations are not likely to be present in large quantities in the products of combustion from motor-car engines, and it was thought unlikely that further attempts to isolate more powerful olfactory stimulants from such products would be successful.

Summary.

The effects of exhaust gases and distillates of sump oil from old motor cars on the activity of *Glossina palpalis* (R.-D.) were briefly investigated, using a method described in the preceding paper. The exhaust gases had no effect on the tsetse flies and the threshold and standard concentrations of vapour of distillates from sump oil required to stimulate the tsetse flies decreased as the boiling point of the fractions increased. This result is consistent with the results of experiments by previous workers using series of homologous pure compounds, and suggests that any very stimulating compound could only be present in very small quantities in sump oil.

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INDIVIDUAL AND GROUP MARKING METHODS FOR FLY-POPULATION STUDIES.

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The use of marked insects as indicators for studies of behaviour, length of life and population numbers has been frequently exploited in recent decades. The development of radioactive-isotope-tracer techniques has given fresh impetus to studies of this type, but has by no means outmoded the alternative method of using visible marks, i.e., paints, dyes or luminescent material. It does not at present appear probable that radioactive labelling will be able to replace the other methods for many types of problem, and the following notes on our experiences with different methods of marking, though relating primarily to the blowfly group, should be of value for ecological studies of mobile arthropods in general.

Marking Methods.

We have made field-scale use of one method for marking individuals and three for mass marking.

Handmarking of individuals.

The flies are immobilised and marked individually. Specially prepared nitro-cellulose paints, incorporating light-resistant pigments, were obtained. We found the scarlet, yellow, light blue and white paints useful for CALLIPHORINAE.

The mark is made with a fine bristle or grass stem, and, if the paint is thinned with acetone, at least ten positions can with care be recognisably marked on the thorax. The paints are classed as slow-drying, but in practice there appeared to be an almost immediate drying of at least the surface of the paint, so that the flies could be pooled as marked without a serious degree of smudging. The marking is readily visible on the living recapture, which can thus be given a further mark and be again released.

Occasionally, if the paint be allowed to thicken, the blob may be held by the thoracic bristles and microchaetae without reaching the surface chitin. Some of these marks may disappear completely, through the bristles breaking away.

Evidence, from experiments in which radioactive flies were paint-marked, suggests that even in large-scale marking experiments the marking failure does not exceed 2 per cent.

Mass radioactive tagging.

The flies are made radioactive by adding ^{32}P to the drinking water or, more reliably, to a sugar solution in the cages in which they were confined.

Labelled flies are detected by screening the wild samples with a Geiger counter. We have killed our samples before screening them, but if recaptures were required alive it should be possible to screen limited numbers at least by applying simple restraint, and large catches by the chill-coma method (see p. 589).

In our experiments the above method of tagging gave good results. Thus, in one experiment, 1,235 wild flies supplied for two days with radioactive sucrose

solution were checked individually for labelling. Only one fly, an example of *Calliphora erythrocephala* (Mg.), registered a nil count, and one of *Phormia terraenovae* R.-D. had a count only slightly above background level; all the remaining flies gave satisfactory counts.

In another experiment, samples of approximately 100 were removed from each of three cages of wild-trapped flies, mainly of *C. erythrocephala*, after two days' feeding, and a sample of 65 from a cage of laboratory-bred flies, containing a mixed population of *C. erythrocephala*, *Lucilia caesar* (L.), *L. sericata* (Mg.) and *P. terraenovae*. They were killed and monitored individually. All were radioactive. However, of 203 examples of *C. erythrocephala* recaptured subsequently in this experiment, and identified from the fact that they carried paint marks, nine gave no appreciable count. If we exclude the possibility of this being due to escapes after marking and before labelling, the result would suggest a 5 per cent. failure of the labelling to take. Lindquist & others (1951) reported 90 per cent. labelling of *L. sericata* and *Phormia* after 24 hours' feeding.

There is a wide scatter of count level when the adult flies are labelled in the above manner. Thus, of the 26 examples of *L. caesar* in the mixed sample of 65 laboratory-bred flies mentioned above, the counts distribution when killed, immediately after feeding, was:

201-300	-400	-500	-600	-700	-800
6	5	7	5	2	1

The mean level differs between species, partly no doubt due to size differences, with their associated differences in the quantities imbibed, but it is also probably related to the habits of the species in captivity. *P. terraenovae* for example is lethargic in confinement. Though a larger species than *L. sericata*, it had a lower mean count.

Even for a single species the scatter about the mean is so great that groups could not be reliably differentiated by feeding them with ^{32}P at different radioactivity levels. We have found, however (Donnelly, unpublished), that if the radioactive phosphate is added to the food of the growing larvae, the count level per milligramme body weight tends to be proportional to the concentration of ^{32}P and this proportional intensity of labelling is maintained through the pupal stage. The scatter in the count level of the resulting adults, at emergence, is largely confined to that associated with weight differences. It should be possible therefore to label larvae with at least two levels of radioactivity which could be distinguished in the adults.

We have used concentrations of ^{32}P ranging from 0.013 to 0.035 millicuries/ml. in 5 per cent. sucrose solution, which was left in the cage for either 48 or 72 hours, and observed no harmful effects subsequently on laboratory-maintained control samples.

The initial counts for those flies fed with the highest concentration, based on samples killed at the end of the feeding period and immediately monitored, were:

<i>C. erythrocephala</i>	(18 flies)	mean 598, range 50-1000
<i>C. vomitoria</i> (L.)	(7 flies)	mean 650, range 250-950
<i>Lucilia</i> spp.	(10 flies)	mean 515, range 200-950

With a feeding concentration of 0.024 mc./ml. the mean initial counts (samples from four cages) were:

<i>Calliphora</i> spp.	(28)	429, range 45-1200
<i>Lucilia</i> spp.	(24)	325, range 60-1200

The relation of count level to dosage in the foregoing examples corresponds fairly well to that obtained by Lindquist & others (1951), who used a concentration of approximately 0.002 mc./ml. (0.020/12 ml.) and obtained counts ranging from 4 to 80 per second.

At the dosage levels which we used, recoveries should be reliably detectable for several weeks; the minimum period can be predicted with reasonable confidence, as follows. The background count from the environment, under normal laboratory conditions, is usually 2 or under. It tends to rise slightly during a period of active labelling and subsequent handling of labelled flies, and is presumably due to distribution of contaminated dust, the track pattern of flies escaped from labelling cages, and similar causes, but should not exceed 5 counts per second. Assuming reasonable facilities, a mean level of 30 counts might, for example, be decided on as the lower limit of acceptable counts. (This is an unnecessarily high threshold.) Suppose a zero-day count level is in the order of 600. The half life of ^{32}P is 14.3 days, the decay curve of radioactive mass exponential. A count level of 30, *i.e.*, 5 per cent. of the initial, is reached in 62 days, through decay.

In addition to this loss, one must allow for loss by excretion; this loss rate would be expected *a priori* to be fixed. A rough estimate of its order of magnitude, based on field evidence of flies recovered over a period of four weeks after release, is 1.5 per cent. per day. This rate is not sufficiently significant to invalidate a cautious estimate based on decay rate alone, which is approximately 5 per cent. Thus, a decrease to, say, 50 due to decay alone could be safely assumed to involve a total decrease to not lower than 30 counts. The curve of decay from an original count of 600 reaches the 50 level (8%) on day 52. Mean counts above 30 could therefore be safely predicted for approximately seven weeks.

The 50 level for initial count levels of 300 and 200 (17 and 25%) are on days 37 and 29, *i.e.*, these initial levels should afford five and four weeks, respectively, of reliable recovery sampling.

Flies labelled as larvae will have lost some of their radioactivity during development, and a further 10 per cent. or so at ecdysis (Donnelly, unpublished). Under normal insectary conditions the prepupal and pupal stages occupy about a fortnight only, so that an initial count in the fully fed larvae in the order of, say, 300 counts per second, should give clearly recognisable labelling for some three weeks of imaginal life.

Mass powdering.

The use of dyes for marking of flies was described by Quarterman, Mathis & Kilpatrick (1954), who used six different colours to trace the dispersal of radioactive flies released at different points. The authors mention that "the flies were dusted", but do not specify the method. They were able to get two extra separable categories, making eight in all, by using fluorescent and non-fluorescent dyes for two of the colours.

We have found mass marking with finely powdered dyes highly successful with the British Calliphorine flies, though the marking is relatively short-lived. The powder is applied by creating a "duststorm" in a small chamber containing the flies. Only a minute quantity is required. A dye-powdered fly is not detectable by ordinary inspection. The killed fly is placed on blotting paper and wetted with a few drops of acetone, which dissolves any trace of powder present, and reveals it as a coloured spot on the paper below the fly. Two dyes on the same fly can usually be separately identified, as two rings of different radius, by this simple paper-chromatography technique.

Ordinary dyes, such as Sudan III, applied, by a powder dispenser of the type used by barbers, to a group of flies through the gauze walls of the cage, give satisfactory "acetone-spots". A series of dyestuffs corresponding to the type of the materials used by the American workers was obtained, and all field tests were made with these.

The dyes are listed below. All but the last three have been tried in the field, and yield satisfactory acetone-spots.

Red. Waxoline Red O.S., Rotor Brilliant Red R.

Yellow. Waxoline Yellow O.S. 150, Auramine Lake Yellow O.150.

Blue. Waxoline Blue A.S., Rotor Blue B.

Green. Waxoline Green G.S.

Purple. Waxoline Purple A.S., Rotor Violet R.

Not tested. Waxoline Yellow I.S., Rhodamine B 500, Methasol Fast Red 3 B.S. The Methasol Fast and the Rotor colours are water-resistant.

With combinations of two different colours, there was usually no difficulty in separating them on acetone test; it is also possible, but not easy, to distinguish between two different dyes of a given colour in this series, and their use in the same experiment is better avoided. Rotor Brilliant Red gave a more distinctively red acetone-spot than Waxoline Red, which was rusty brown and might be confused with Rotor Violet. In a set of colours containing green and blue, the slightly purplish blue of Waxoline Blue was more easily distinguishable from the green than was the rather greenish blue of Rotor Blue.

The duration of the dye mark is not comparable to that of paint marks or radioactive label; in caged flies the acetone spot was faint or absent after two weeks, the occasional fly being negative after one week or so.

Powdering of radioactively tagged flies.

In this extension of the powdering method the flies to be powdered are first made radioactive, to allow of a quick separation of marked flies prior to testing them with acetone for dye colour. It avoids the necessity for acetone-testing of the entire catch, and is useful where the conditioning of the flies, unavoidably associated with their radioactive tagging, is unimportant. The combination of powder-dyeing of adults with labelling in the larval stage has proved a useful tool for experiments where it is required to mark separately different groups of radioactive flies, and where the flies to be used must be allowed to emerge in outdoor environments and not be subsequently confined for long periods for radioactive labelling.

Self-marking with fluorescent material.

A method of allowing flies to mark themselves as they emerge, thus avoiding any conditioning of the teneral flies by cage-confinement for marking, deserves mention, though we have so far tested it only on a laboratory scale. The method, originated by K. R. Norris for Australian blowflies (Australia, 1952), consists in placing a layer of material impregnated with a fluorescent powder over the soil in which the pupae are developing. The particles of dust on the everted ptilinal sac are concentrated in the ptilinal suture when the sac collapses, and are to some extent protected there by the bristles. Examination of the face of recaptured flies under ultra-violet light reveals the characteristic fluorescence of the material used.

So far we have had successful results with commercial anthracene, which is ground up in the first place with very finely sieved silver sand to give a five per cent. dilution, this being subsequently diluted with ordinary silver sand to one per cent. or less. Many of the sulphide phosphors available as proprietary preparations, though giving good marking, are photo-labile under conditions of outdoor exposure. The soluble fluorescent substances, such as Fluorescein, Rhodamine, etc., have the disadvantage that they fluoresce only in solution, and it is necessary to expose each fly to a steam jet or otherwise to dissolve the retained dye before examining it. The method deserves further study, because of its obvious theoretical advantages.

Indications for use of the different methods.

Where individual flies of a marked release-batch must be distinguishable, *e.g.*, in small-scale ecological studies, or where it is intended to re-mark and release again any recaptures made, the method of individual marking is necessary. It is, however, very tedious, and limits the number which can ordinarily be marked. There is often a high mortality in the marked flies, and a proportion may be incapacitated by the paint, *e.g.*, when a badly placed or over-large blob bridges the space between the thorax and head, immobilising the latter, or when the inner margin of the wing gets stuck to a paint mark on the right or left thoracic margin.

Where marked flies are required to be distinguishable merely between groups, *e.g.*, between releases on different days, or in different areas, and where recoveries are not being identified and released again, a mass marking with different dyes is appropriate. An example is the release of laboratory-bred groups, of known composition.

Where it is undesirable that wild-caught flies be conditioned in any way by confinement in cages, simple powdering is indicated. If each trap-catch is to be dealt with at the site of the trap, with immediate release, the method provides an excellent and simple means of marking flies in their own territory, *e.g.*, for study of movements and groupings on a microgeographic scale.

The one serious limitation to the method, apart from the necessity for killing recoveries, is the need for individual acetone-testing of each fly in the post-release samples. It is therefore an advantage to label all the marked flies, so that all post-release samples may be subjected to a rapid preliminary screening, wherever the experimental conditions permit. Examples are: when the wild catches can be confined for two days in cages, when there are not large numbers of small groups to be separately labelled, or when laboratory-bred material is being used.

Radioactive labelling alone may sometimes be all the marking required, as for example when only a single category of marked flies is involved and recaptures are not being released again.

Handling Techniques.

Immobilisation of flies.

In immobilising flies for marking one is faced with the difficulty that a trap-catch has to be restrained long enough for the whole catch to be identified and marked, and the catches of individual traps may exceed 1,000 flies. Obviously, an anaesthetic could be used only on small numbers at a time, *i.e.*, a heavy trap-catch might require to be subdivided into several confined lots, a complication which with large numbers of trap-catches to be attended to could prove impossibly difficult. Even with small catches, the variation in the time taken for anaesthesia might result in an irregular and unpredictable mortality.

In our blow-fly work we made use of chill-coma, so that the pace of marking would not have to be rushed by the threat of imminent recovery of those still to mark, and interruptions of the marking would be possible, the flies merely remaining for a little longer in cold-torpor. We use a double-walled tank, lagged on the outside, and with ice-water mixture between the walls. The copper inner container is 30 in. long by 30 in. deep, and only broad enough to take a trap. It is divided by a vertical partition, each half having its own lid. The introduction of warm air incident to removal of a trap is thus confined to half the tank. The temperature in the lower part of the tank varies from 0° to 5°C. according to the frequency of opening. It was found advisable to load the compartments not more than half full, otherwise the flies in the uppermost traps were not sufficiently chilled.

The traps on being brought in from the field are placed in a cold store, at a temperature of approximately 5°C., and left for about one hour. The two

compartments of the chilling tank are then loaded with 10 or 12 traps each. After half an hour the flies are completely comatose. The traps are removed, as required, from one compartment until it is empty; it is then reloaded with a further 12 traps from the cold store, and work is started on the second compartment. By this means large numbers of traps can be dealt with at the rate of some 20-25 per hour.

The chilled flies are marked in a special "cold tray", consisting of a lagged copper box about 15 x 10 in. by 6 in. high. About two inches above the bottom is the working floor, consisting of a removable shallow tray, under which is a sliding drawer containing a slab of ice. Shuttered armholes in the sides of the box allow manipulation, observation being through a perspex lid.

The cold tray contains the paint, bristles, and a "recovery jar",—a glass vessel with a truncated cone as lid. The flies are dropped into this as marked, and though often recovered sufficiently to walk they are prevented by the cone from escaping.

The chilled flies are transferred to the cold tray, and the operator's hands are introduced through the rubber shutters of the armholes. After a time the heat loss from the hands may cause the flies to become fairly active, but generally if the trap-catch is not excessively large, marking can be completed while the flies are relatively quiescent. The recovery jar is then replaced by an empty one, and, after a minute or two for the temperature to readjust itself, the cold tray is ready for the next lot.

With a heavy marking programme, we have found it advantageous to separate the identification from the marking. The identification tray has a number of cavities in it (the housewife's pastry-cake tray serves admirably for the purpose). The tray rests in a deeper tray of similar size. To prepare the tray, water is added to the lower tray until the cavities of the upper are at least partially immersed, and then frozen so that the cavities are embedded in ice. Two or three such are prepared, and, like the drawers containing ice slabs in the cold trays, are renewed as required, the partially thawed tray or sliding drawer being returned to the freezing machine for re-freezing.

A number of petri dishes or similar glass covers are kept on ice. The chilled trap-contents are emptied on to the identification tray, and apportioned roughly between the cavities, which are then separately covered by the glass covers. Thus, only a small group need to be exposed to the room temperature while being identified, and any movement which may be shown soon ceases after identification of the group is completed and the cover replaced. When all the flies in the tray have been identified they are transferred to the cold tray for marking.

As an alternative to physiological immobilisation we have used a simple restraint method. The traps are opened inside bags of which one side is a net of one-eighth-in. mesh. The flies are identified through the net, and held immobile for marking by pressing the net down by a finger on each side of the fly. The method does not allow of precise positioning of the mark, nor of identification of the more difficult species. Where these objections are not important it is a useful and expeditious method, and has the advantage that it can be used in the field, *i.e.*, trap-catches can be marked and released on the spot. Cragg & Hobart (1955) successfully used a similar method in their marking experiments with *L. caesar*.

Mass powdering.

A small pinch of dye powder is placed in a metal container, such as a biscuit tin. The flies to be marked are transferred to the tin, the air in which is then agitated by means of a small hand-blower and a tube. Dusting the flies in the actual trap was tried, but had to be discontinued because of the difficulty of decontaminating the trap before setting it up again.

One pinch of powder is sufficient for several dustings, so it is convenient to keep a separate dusting tin for each dye.

In testing for presence of dye, the dead fly should be laid on its side, as the last traces of dye are likely to be found in the protected crevices thus exposed, *e.g.*, around the halteres and squamae. The flies should not be laid out closer than one inch apart. The colour is concentrated in the perimeter of the circle of wetting, the general area of the stain showing a fainter intensity. If the flies are too close there is a danger of the perimeters of the circles intersecting. For the same reason, no more than two or three drops of acetone per fly should be used. If the acetone-spot is faint, through loss of dye from the fly or from inadvertent use of too much acetone, the colour can be concentrated by applying acetone outside this ring, and thus carrying the colour inwards on a backwash, as it were.

Only white blotting paper should be used; the two yellow dyes, for instance, gave unreadable spots on any other than a pure white paper.

Radioactive labelling.

The ^{32}P is obtained, by authorisation of the Medical Research Council, directly from the Atomic Energy Research Establishment, Radiochemical Centre, Amersham. Our samples were usually five millilitres, having a concentration of either one or two mc./ml. of carrier-free labelled phosphate in aqueous solution. The sample as received has a stated specific activity with reference to a stated date, which may be subsequent to the date of receipt; this specific activity is corrected for any interval between supplier's and user's day-zero by applying the appropriate correction for decay.

Dilutions and transfers were made by means of syringe pipettes, the operator wearing rubber gloves and laboratory protective clothing.

The method of feeding the flies was as follows: a 5 per cent. sucrose solution was prepared as a diluent, and the required quantity of PO_4 concentrate was added to this. Twenty or thirty ml. of the diluted ^{32}P was pipetted into a 3×1 -in. vial, which was inverted in a petri dish containing a sheet of filter paper. Two to four vials were used per cage. Other drinking water was withheld, to ensure that the flies drank the radioactive solution. Small holes were punched in the filter paper to increase the amount of free liquid available. The vials were generally half to three-quarters empty within a day, and were topped up as necessary, sometimes with plain sucrose solution, on other occasions with a labelled solution.

The concentrate and contaminated equipment is handled as little as possible. We confine all manipulations involving the radioactive material to a side room which can be locked from the rest of the laboratory, communication being through an outer door. Thus accidental escapes, or wild flies contaminating themselves, are unable to pass on immediately into the main laboratory and contribute to "background" contamination by alighting on, for instance, note-paper, or on fabric, with feet or proboscis still damp with labelled solution.

The handling is done on enamel trays, on a special bench with sides and lid so that the materials and equipment when not actually in use can be shut away without the necessity for handling them. Instruments and small contaminated vessels are stored under water in a ten-gallon earthenware jar. Cages and equipment in which flies have been labelled are stored in an attic until they have lost their radioactivity.

Detection of the beta radiations of the ^{32}P is by means of a beta-gamma monitor. Both mains and battery-operated models are available. We use an Airmec type 1021B mains model incorporating a type B.12 Geiger-Muller tube which is housed in a duralumin probe unit. In use, the probe housing is so clamped that the tube window, which is exposed through a 5×1 -in. aperture in

the casing, is a fixed 3 cm. above the specimen holder. The holder is a plywood frame consisting of guides and a sliding tray, on which the fly is placed and slid under the window. Any radioactivity present is registered on the monitor as a count rate (*i.e.*, of disintegrations per second). The tray floor is covered with blotting paper, which is checked for contamination after each positive reading, and renewed if registering more than one count per second.

According to the makers, this tube is insensitive to light, but after one series of confusing results we found that our instrument had become sensitive to sky radiation. It is advisable therefore to take precautions to avoid the tube being exposed to direct lighting more than is necessary.

Summary.

Four different methods of marking insects are described in detail. Although they have been applied by the present authors only to the British CALLIPHORINAE, they should be of value in ecological studies of mobile arthropods in general. These methods are: individual marking with paints, mass powdering with dyes, radioactive labelling with ^{32}P , and a combination of the last two. A fifth method, in which the emerging fly labels itself with fluorescent dust, is briefly described. The circumstances affecting the choice of method are outlined.

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THE EFFECT OF EXTREME TEMPERATURES ON DIFFERENT STAGES OF *AËDES AEGYPTI* (L.).

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22.

Several investigators have studied the effect of extreme temperatures on the various stages of *Aedes aegypti* (L.): Macfie (1920), Bliss & Gill (1933), Hatchett (1946), Woodhill (1949), Farid (1949) and Gander (1951).

The purpose of the present investigation was to supplement these data by a study of the effect of extreme temperatures on eggs, larvae and pupae in water and on eggs and adult females in air at various relative humidities.

Effect on Larvae and Pupae.

Materials and methods.

In experiments performed at low temperatures, groups of 100 larvae of the same stage were kept for various periods of time at constant temperatures in incubators (60 × 38 × 39 cm.) in a cold room (2–4°C.), the temperature inside the incubators being controlled, by a bimetallic thermostat, to $\pm 0.5^{\circ}\text{C}$. To maintain the larvae at 0.5°C . they were placed in Erlenmeyer flasks inserted in ice fragments. At the end of each experimental period (6–384 hours), the larvae were transferred to water at 28°C ., and the mortality was determined after 24 hours. The survivors were reared to the adult stage and counted.

The experiments with pupae were carried out with both young and old individuals, as it was found (as will be seen later) that the temperature resistance of the pupae varies considerably with their age. "Young" pupae are defined, for the purpose of the present study, as those which had pupated within the previous half-hour, and "old" pupae as those at least 36 hours old. As it is difficult to obtain large numbers of young pupae, the experiments with this stage were carried out with groups of 25.

The experiments at high temperatures were carried out using a water bath. Larvae of each stage in groups of about 100, and young and old pupae in groups of 25, were introduced into a glass cylinder (8 cm. × 5 cm. diam.) which was closed at one end with linen cloth. The larvae or pupae were filtered out on the inner side of the linen cloth. The cylinder with the larvae or pupae was introduced immediately into the water bath, so that the water about half filled the cylinder. At the end of the experimental period (3–960 minutes), the tube was removed and introduced into water at 28°C . Mortality was determined after 24 hours. The time required for 50 per cent. mortality (T50) was determined by the method of Reed & Muench (1938). The survivors were reared to the adult stage and counted.

Results.

Results are given in Tables I and II. The numerical data that were used to calculate the T50 in these and all the following experiments are deposited in the Archives of the British Museum (Natural History).

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In the case of larvae, there was little further mortality amongst those surviving 24 hours after removal from the test temperature. In the tests of pupae, however, there were some cases in which many of those surviving after 24 hours failed to reach the adult stage. The T50 for pupae was therefore determined from the number of adults obtained.

TABLE I.

Number of hours' exposure to low temperature required to give 50 per cent. mortality (T50) of immature stages of *Aedes aegypti*.

Temp. °C.	Larval stage				Pupae	
	1st	2nd	3rd	4th	Young	Old
0.5	18.3	15.4	17.7	14.9	31.0	12.1
4	23.7	21.6	22.1	24.0	60.3	27.4
8	90.2	75.8	81.3	85.3	217.5	74.8

It can be seen that young pupae are more resistant than old ones at both high and low temperatures. In fact, young pupae were the most resistant of all immature stages. It appears that the four larval stages, and the old pupae, have about the same order of resistance, as regards high and low temperatures.

TABLE II.

T50 (in minutes) of immature stages of *A. aegypti* at high temperatures.

Temp. °C.	Larval stage				Pupae	
	1st	2nd	3rd	4th	Young	Old
41	153.6	163.8	236.4	207.6	376.5	264.4
43	68.8	68.8	66.0	55.9	64.7	39.5
45	9.9	9.6	10.5	8.8	12.3	8.1

Effect on Adults.

Materials and methods.

In studying the effect of extreme temperatures on the adults, another factor, the relative humidity (R.H.) comes into play. The experiments were, therefore, carried out at various relative humidities, and at different temperatures: low (0.5, 4, 8 and 12°C.), medium (28°C.), high (35°C.) and very high (40, 41, 43 and 45°C.).

Groups of 50 four-day-old females previously held at 28°C. and 60 per cent. R.H., were maintained in hermetically closed jars (16.5 cm. long × 7.5 cm. diam.) at the desired temperature in incubators as described above. At the bottom of each jar was placed another (5 cm. high × 3 cm. diam.), closed with a net cloth and containing either water, saturated solutions of K_2CO_3 or KCl, or concentrated H_2SO_4 , giving 100, 43-47, 83-86 or 0 per cent. R.H., respectively. The adults had no food or water during the experiment. After the experimental period

(6-48 hours at low, 6-240 hours at medium, 3-78 hours at high temperatures), the jars were opened in cages maintained at 28°C. and 60 per cent. R.H., and containing water and honey, and the mortality examined after 24 hours.

At the very high temperatures (41-45°C.), groups of 50 four-day-old females were placed in a wire-screen tube (18 cm. long \times 3 cm. diam.) which was then introduced, through a suitable hole, into an incubator, as described previously. The incubator contained a fan which kept the air circulating. At the end of the experimental period (1.5-60 minutes) the mortality was determined as above. At these temperatures no attention was paid to the relative humidity, as it was found (see below) that by 40°C. this had ceased to exert any effect.

Results.

Results are given in Table III and Table IV, column 6. At 0.5°C. and 40°C. the relative humidity had no effect, but between these extremes, the mortality was inversely proportional to it.

TABLE III.

T50 (in hours) of adults (\varnothing \varnothing) of *A. aegypti* at various temperatures and humidities.

Temp. °C.	% R.H.			
	0	45	85	100
0.5	9.9	10.2	8.2	9.7
4	20.2	21.0	33.0	48.7
8	13.0	23.0	26.4	32.0
12	4.9	6.5	14.0	25.0
28	15.4	31.9	32.3	104.7
35	4.3	12.9	18.8	48.3
40	0.6	0.7	0.7	0.6

It was surprising to observe that at 8°C. the mortality tended to be higher than at 4°C., and at 12°C. higher than at 8°, at all the humidities used. This appears to be related to the fact that at 8°, and still more so at 12°, there was some activity (slight movements of the legs, and buzzing) not observed at lower temperatures (0.5-4°C.).

The activity of the mosquitos at 12° was compared with that at 28°C. For this purpose, 50 females were kept at each temperature. After two hours, the numbers of females showing activity (crawling, movements of legs or flight) were recorded every 30 seconds for 10 minutes. The results were as follows:—

At 28°C.: 1, 2, 3, 2, 1, 1, 3, 3, 2, 2, 3, 1, 2, 2, 2, 4, 1, 2, 3, 2.

At 12°C.: 30, 33, 28, 25, 25, 30, 34, 32, 26, 29, 35, 22, 29, 30, 35, 32, 29, 20.

It is an interesting fact that at the lower temperature, when the activity might be expected to be smaller, it is in fact much greater.

Effect on the Eggs.

Materials and methods.

The method of determining the effect of extreme temperatures on eggs in water was the same as that used with larvae, and in air at various humidities, the same as that used with adults. At the end of the experimental periods

(6-960 minutes at high temperatures, and 4-32 days at low temperatures in water; 0.25-32 days at high temperatures and 2-32 days at low temperatures in air), the eggs were transferred to water at 28°C. with the addition of yeast as food. After 24 hours, the eggs were dried for about 24 hours, and then replaced in water at 28°C., with the addition of food. This procedure was repeated several times, until no more larvae hatched. The proportion hatching was then determined. Each experiment was carried out with about 1,000 eggs. Of the larvae that hatched, about 50 were reared to the 3rd or 4th stage, when the percentage mortality was determined. It was found that larvae reaching this stage will nearly all reach the adult stage.

Results.

Results for eggs exposed at extreme temperatures in water are given in Table IV, column 2, and Table V, column 2. Larvae which hatched after exposure of

TABLE IV.

T50 (in minutes) of the various stages of *A. aegypti* at high temperatures.

Temp. °C.	Eggs in water	Larvae	Young pupae	Old pupae	Adults
41	624.6	190.3	376.5	264.4	32.8
43	117.4	64.9	64.7	39.5	16.9
45	27.6	9.7	12.3	8.1	6.8

The values for larvae are the means of the values for the four stages given in Table II.

the eggs to extreme temperatures developed normally.

In experiments at extreme temperatures (35 and 40°C.; 0.5, 4, 8 and 12°C.) as well as at 28°C. (control) in air at various humidities as used previously, the results were as follows:—

35°C. and 0 per cent. R.H., T50 = 2.8 days

40°C. and 0 per cent. R.H., T50 = 1.4 days.

At all the other temperatures and humidities used, for periods up to 32 days, the eggs hatched normally, except at 28°C. and 100 per cent. R.H. In this case only 12 per cent. hatched after 16 days and 2 per cent. after 32 days, the reason being that the eggs became covered with fungi which developed on the

TABLE V.

T50 (in hours) of the various stages of *A. aegypti* at low temperatures.

Temp. °C.	Eggs in water	Larvae	Young pupae	Old pupae	Adults at 100% R.H.
0.5	394	16.6	31.0	12.1	9.7
4	446	22.8	60.3	27.4	48.7
8	523	83.1	217.5	74.8	32.0
12					25.0

The values for larvae are the means of the values for the four stages given in Table I.

paper to which the eggs adhered. The mycelium also penetrated the eggs and killed them.

These experiments show that the resistance of eggs to extreme temperatures is far greater in air than in water.

Discussion.

The responses of the various stages to extreme temperatures are summarised in Tables IV and V.

The order of resistance to high and low temperatures was as follows: eggs, young pupae, old pupae and larvae, adults. It can be seen that the differences between the various stages as regards their resistance were far greater at low than at high temperatures.

The lower resistance to extreme temperatures of old as compared with young pupae may be due to the fact that an old pupa is an almost mature adult (the least resistant of all stages) enclosed in the pupal case, whereas a young pupa is practically still a larva protected by a hard outer covering.

As far as adults are concerned, it seems that several additional factors come into play in determining their sensitivity to extreme temperatures under conditions of starvation: the loss of water by evaporation; the greater activity at certain temperatures, associated with faster metabolism and consequently increased loss of water (Mellanby, 1934); and the exhaustion of food reserves. In those cases in which mortality, at a given temperature, is independent of the humidity, the mortality is probably a direct effect of the extreme temperature or is due to the exhaustion of food reserve (if the experimental period is sufficiently long). If, on the other hand, the mortality, at a given temperature, rises with decreasing humidity, it may be assumed that the mortality is due to loss of water by evaporation (Mellanby, 1932).

At 0-5°C., the mortality seems to be due to the effect of cold only, since humidity has no effect. At 4°C., where the humidity has a marked effect, loss of water seems to be an additional factor. At 8°C., a third factor seems to come into play, the slight activity (buzzing and movements of legs) which increases the loss of water. At 12°C., the activity is greatly increased and may cause the high mortality. At 28°C., where the humidity has a very marked effect, one can ascribe the mortality to the loss of water alone. In any event, the mortality at this temperature was not caused by the exhaustion of the food reserves, as the mosquitos at 100 per cent. R.H. lived longer than at lower humidities. At 35°C., the mortality is much greater than at 28°C., as the evaporation at this temperature is much faster. At 40°C., the mortality seems to be caused directly by the heat, since variation of humidity has no effect, and the value of the T50 is relatively small.

The high mortality of the eggs at 35 and 40°C. and 0 per cent. R.H. is very probably due to the rapid drying of the eggs.

Summary.

Earlier investigations of the effects of extreme temperatures on different stages of *Aedes aegypti* (L.) were supplemented by studies of eggs, larvae and pupae in water, and of eggs and adult females in air at various relative humidities.

Larvae of the same stage, young pupae (defined as those less than half-an-hour old) and old pupae (at least 36 hours old), in groups of 100, 25 and 25, respectively, were kept for various periods at low temperatures in incubators, or at high temperatures in glass cylinders closed at their lower ends by cloth filters and inserted into water baths, and then transferred to water at 28°C., and the mortality determined from the numbers surviving 24 hr. later (in the case of larvae) or becoming adult (in the case of pupae). Eggs in batches of 1,000

were similarly treated, except that periods of 24 hr. in water at 28°C. with yeast as food added were alternated with 24-hr. periods of drying, and mortality determined on the number that hatched and survived to the third or fourth stage. Adult females (four days old) were exposed in groups of 50 for various periods in jars over appropriate solutions giving a complete range of relative humidities and kept at low, medium and high temperatures. Eggs in batches of 1,000 were subjected to the same treatments. The adults were kept at 28°C. and 60 per cent. R.H. before treatment and for 24 hr. afterwards and mortality then determined. Mortality of eggs was determined after alternate 24-hr. periods of wetting and drying at 28°C., as in the case of eggs treated in water. The results of all the experiments were expressed as the exposure-times required to give 50 per cent. mortality (T50), calculated from the observed data.

The resistance to extreme temperatures (0.5, 4, 8, 41, 43 and 45°C.) of the various larval stages and old pupae was about the same. Young pupae were more resistant than old ones.

The resistance of adults to very low and to very high temperatures (0.5°, and 40°C. or over) was unaffected by humidity, the temperatures alone being lethal; at the other temperatures examined (4, 8, 12, 28 and 35°C.) relative humidity was the more important factor. The value of the T50 was lower at 12° than at 8° and at 8° than at 4°C., probably because of the greater activity at the higher temperature. Activity was, furthermore, much greater at 12° than at 28°C.

The resistance of eggs to extreme temperatures was much lower in water than in air, in which at 0.5–28°C. and 0–100 per cent. R.H. the eggs hatched normally, except at the highest temperature and humidity, when fungi developed and killed them.

The order of resistance of the various stages to extreme temperatures was as follows: eggs, young pupae, larvae and old pupae, and adults. The differences between stages as regards their resistance were much greater at low than at high temperatures.

Acknowledgements.

The writer is indebted to Professor Dr. F. S. Bodenheimer for his interest throughout this work, and to Mrs. H. Jordan for excellent technical help.

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THE RESTING SITES OF *GLOSSINA SWYNNERTONI* AUST. IN THE WET SEASON.

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26.

(PLATE XV.)

A sample of a population of *Glossina swynnertoni* Aust. obtained on a fly-round (Buxton, 1955, Appendix 5) always contains a low percentage of female flies. In catches made on five fly-rounds in Block 9, Shinyanga,* twice monthly during January and February 1956, the overall percentage of females amongst non-teneral flies was 3.4 (a fly is referred to as non-teneral from the time of its first meal onwards). Moreover, nearly all female flies caught on a fly-round are hungry.

Assuming that the estimates (Jackson, 1949) of one month for the average life of male flies, and of the same period for the time needed for a female to produce two larvae, are approximately correct, then since male and female flies emerge in equal numbers from puparia, it appears that female flies must live at least as long as males, and therefore must exist in much larger numbers than those revealed by fly-round catches. Further, it is unlikely that almost the whole population of females is composed of hungry flies. It is therefore clear that fly-round catches do not give a representative sample of a population of *G. swynnertoni*, and a search has been made for resting flies in order to discover the missing elements in the population, and to find out where flies spend their inactive periods.

Comparison of records of previous observations on resting tsetse flies shows that there are variations from one species to another in the situations chosen as resting places. *G. fusca* (Wlk.), *G. medicorum* Aust. and *G. longipalpis* Wied. rest on the trunks of thin saplings and vertical creeper-stems, with the head pointing downwards (Nash & Davey, 1950); in the Gold Coast, Morris (1934) obtained *G. longipalpis* from bushes and undergrowth. *G. tachinoides* Westw. and *G. m. submorsitans* Newst. rest on the trunks of larger trees about half an inch from the ground, during very hot, dry weather, in northern Nigeria (Nash, 1937); *G. tachinoides* has also been seen resting on the undersides of branches (Moiser, 1912). Lamborn (1916) found females of *G. morsitans* Westw. resting in crevices of large trees, and males "in more obvious positions". whereas Nash (1952) records that both sexes rest on horizontal or vertical surfaces of trees. Jackson (1946) observed newly emerged individuals of *G. morsitans* and *G. swynnertoni* perched on the underside of small branches 6-12 feet above the ground. Jewell (in East Africa High Commission, 1951, p. 21), in Ankole, found that *G. morsitans* could be shaken from small *Acacia* trees during the early-morning period when flies were inactive; on another occasion (personal communication) he found experimentally fed individuals of *G. swynnertoni* on the underside of level branches in Block 9, Shinyanga. Swynnerton (1921), using caged examples of *G. morsitans*, *G. brevipalpis* Newst. and *G. pallidipes* Aust.,

* Block 9 is an area of thornbush country 50 square miles in extent, situated in the Lake Province of Tanganyika Territory.

observed that flies rested on a background which harmonised with their own body colour. He also records that crevices were used as resting places. Van den Berghe & Lambrecht (1954) found *G. brevipalpis* resting on tree trunks and lower branches in the secondary thicket vegetation of the Malagarasi River (Urundi, Belgian Congo). *G. brevipalpis* has also been found in bushes, along the bank of the Kagera River, Bukoba District, Tanganyika (Jewell in East Africa High Commission, 1951, p. 21).

These observations show that the type of resting place chosen is not always constant even within a species; it is probable that the variations are due to differing climatic and vegetational conditions, and possibly changes in the habitats and positions chosen for resting usually correspond with seasonal changes.

In the investigation reported here, counts were made of resting individuals of *G. swynnertoni* found during the short rainy season of 1956 in an area of hard-pan of about one-eighth of a square mile, near the Mwakilendya River in Block 9, Shinyanga; part of this area is shown in Plate XV, fig. 1. Collections were made on the dates indicated in Table I by searching at random the hard-pan vegetation.

TABLE I.

Percentages of females in catches of *G. swynnertoni*.

Date (1956)	% ♀♀ in total N.T.* catch		Total number of flies caught	
	Active	Resting	Active	Resting
Jan. 19	2	83	229	6
21	12	18	8	13
23	9	30	11	10
24	2	28	40	18
27	No data	36	None caught	14
28	"	29	"	7
31	"	33	"	3
Feb. 1	0	45	88	27
3	2	30	60	20
6	4	30	53	20
8	0	9	39	22
9	2	24	47	45
10	4	10	95	50
13	2	28	51	39
15	5	23	102	31
16	7	24	132	29
18	3	26	65	38
20	9	11	57	84
21	4	28	67	53
22	13	43	77	51

* N.T. = non-teneral.

The flies were nearly all resting on the undersides of branches of small trees, with the long axis of the body parallel with that of the branch, and the head pointing up the slope of the branch when this was not horizontal (see Pl. XV, fig. 2). The branches chosen sometimes had flakey bark, but were not particularly rough, and no flies were found in crevices.

Some flies apply themselves much more closely to the bark than others, and possibly this has some significance in concealment or water conservation. In Plate XV, figs. 3 and 4 show two extremes, fig. 3 showing a fly immediately after alighting on the branch.

The height of the resting places varies, lowest and highest recorded being about three and fifteen feet from the ground, respectively. Flies may rest at greater heights but it would be impossible to detect these with present methods.

Various species of tree harbour resting flies, but most were found on *Lannea humilis*, of which Plate XV, fig. 2, shows a typical specimen. This tree usually has several fairly smooth, unobscured branches within a distance convenient for searching, and possibly provides favourite searching, rather than resting, places. Other trees on which resting flies have been found are:—*Commiphora schimperi*, *C. fischeri*, *C. subsessilifolia*, *C. ugogensis*, *Dalbergia melanoxylon* and *Acacia drepanolobium*.

It is possible to recognise the type of tree which is likely to harbour resting flies, and possibly shape is the important factor for the fly. The trees chosen usually have lower branches that are not obscured by branches or leaves at the sides and below, but that are shaded by leafy branches above.

The composition of catches of resting flies is influenced by the fact that active flies follow the searchers and settle on trees near by. In view of this, Nash (1952) used a herd of cattle to attract active flies and so remove them from the area that was being searched. Lamborn (1916) collected flies from trees during the heat of the day, when practically all the flies were inactive. The method adopted in the present investigation has been to catch any active flies seen whilst searching for those at rest and to record each category separately. Flies were collected from about 8.30 a.m. to 1.30 p.m.; as the flies remained active throughout the day it was not possible to use Lamborn's method. In this way some active flies undoubtedly escape the catchers and settle on trees near by. They are then caught and included in the catch of resting flies, and since they are presumably mostly males, increase the percentage of male flies to an unknown degree.

For catching purposes, a 6 × 1-in. glass tube was found to be very useful, as the use of a net was often hampered by inconveniently placed branches. In addition, the method is much quieter; flies can often be caught without causing their neighbours to fly away, which would be impossible using a net.

The number of flies to be expected on any one tree is small. In Block 9, Shinyanga, during January and February 1956 the apparent density was 161. That is, the average number of non-teneral male flies caught over 10,000 yards of fly-round was 161. The standard availability (Jackson, 1953) of *G. swynnertoni* is about 10 per cent.—that is, the number of flies caught over 10,000 yards of fly-round represents 10 per cent. of those present in one square mile. The density of non-teneral male flies in Block 9 during January and February 1956 was therefore about 1,610 per square mile. Assuming that females are twice as numerous as males, and that a hundredfold concentration occurs in hard-pan areas, only one female could be expected per 10 square yards.

Hard-pan areas have long been recognised as important in the life of *G. swynnertoni*; for instance, concentrations of male flies are observed there. The fact that flies of both sexes are found resting in hard-pan areas strengthens this conclusion, and it has been suggested that knowledge of the resting places may be put to practical use in anti-tsetse measures. Wilson (1953) eradicated *G. palpalis fuscipes* Newst. by spraying residual insecticides on vegetation in only part of its habitat. A similar method is envisaged for *G. swynnertoni*, in this case the spraying of resting sites within hard-pan areas.

Results.

The percentages of females found among active and resting flies are compared in Table I, and other data are set down in Table II and fig. 1. Inconsistencies in the numbers given in Tables I and II are due to the fact that in some cases

information about flies was incompletely recorded; such flies have been excluded from the Tables where necessary.

There was a considerable difference between the resting population and the active one. The former showed a comparatively high percentage of females, a

TABLE II.

Composition of catches of *G. swynnertoni*.

	For active flies	For resting flies
♀♀ (N.T. and T.)** as % of total catch	7.5 ($\frac{138}{1846}$ *)	28 ($\frac{184}{665}$)
♀♀ (N.T.) as % of total non-teneral flies	4.5 ($\frac{45}{991}$)	25 ($\frac{146}{578}$)
♀♀ (T.) as % of total female flies	62 ($\frac{74}{120}$)	20 ($\frac{34}{174}$)
♀♀ with blood as % of non-teneral ♀♀ (equivalent of Stages I and II in ♂♂)	6.3 ($\frac{3}{48}$)	59 ($\frac{87}{146}$)

* Numbers in brackets show numbers of flies on which percentages are based.

** T. = teneral. N.T. = non-teneral.

high proportion of Stage-I (Jackson, 1933) male flies, and a considerable number of females corresponding to Stages I and II of male flies. Active female flies were largely teneral.

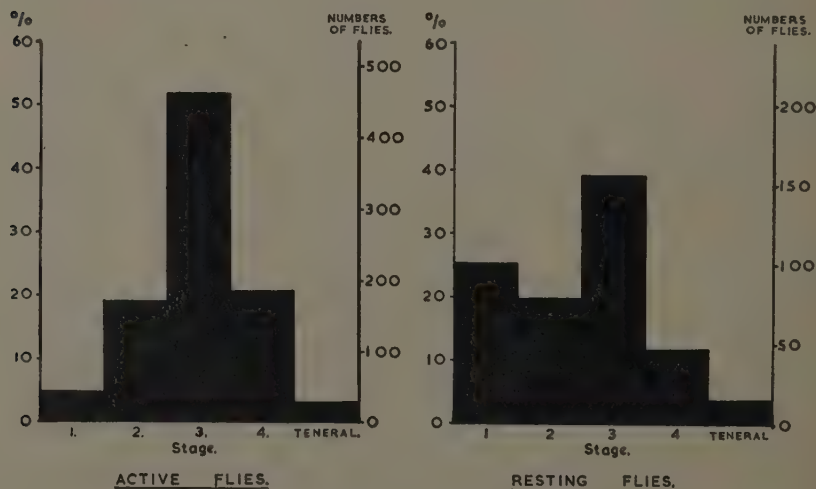


Fig. 1.—Distribution of hunger stages in catches of males of *G. swynnertoni*.

The overall percentage of females amongst non-teneral resting flies is 25. Though this does not nearly approach the expected figure for the percentage of females in nature, it is much higher than the corresponding figure (4.5 per cent.) for active flies caught at the same time and in the same area as the resting flies. It is thought that the discrepancy would be much reduced if the active flies that settle on trees could be excluded from the catch of resting flies.

Summary.

The biased nature of the catches of *Glossina swynnertoni* Aust. made on fly-rounds at Shinyanga, Tanganyika Territory, which show an unduly low proportion of females, nearly all of which are hungry, and lack of knowledge of the fly's habits when inactive, has led to a search for resting flies.

Previous recorded observations on resting tsetse flies are briefly reviewed. These show that the resting sites chosen differ according to species and even within a species. Differences of the second kind probably reflect seasonal changes.

G. swynnertoni was found to rest, during the short rainy season at Shinyanga, on the underside of branches of small trees in hard-pan areas. Resting flies were easily caught with 6 × 1-in. glass tubes.

Samples thus taken differed in composition from those taken on a fly-round, mainly in having higher percentages of female and Stage-I flies.

Knowledge of the fly's resting habits may be of use in anti-tsetse measures using residual insecticides.

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I wish to thank Dr. J. P. Glasgow for guidance and valuable suggestions, Dr. E. Bursell for an introduction to the field work, and Mr. J. M. B. Harley, who kindly took the close-up photographs of resting flies.

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FIG. 1. View of an area of hard-pan near the Mwakilendya River in Block 9, Shinyanga, taken in the short rainy season (Jan. 1956). In the middle distance is a group of *Lannea humilis*.



FIG. 2. Observer pointing to the resting site of a female of *G. swynnertoni*. The tree is *Lannea humilis*.

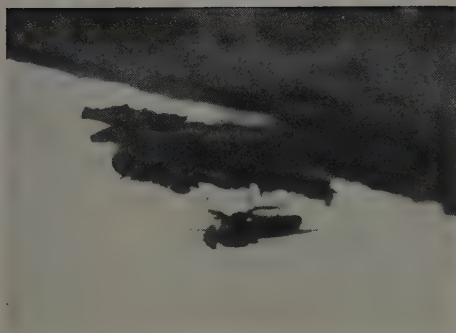


FIG. 3. A fly photographed immediately after alighting.

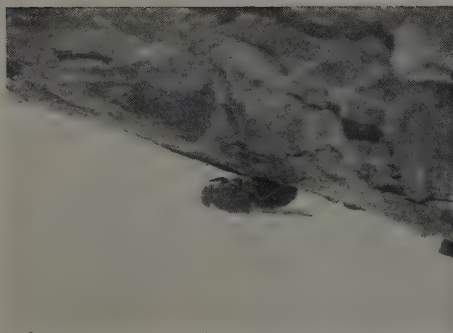


FIG. 4. A fly closely applied to the branch under which it is resting.

(Figs. 3 & 4 from photographs by J. M. B. Harley.)

NOTES ON THE *SIMULIUM NEAVEI* GROUP OF SIMULIIDAE
WITH PARTICULAR REFERENCE TO *S. NYASALANDICUM*
AND *S. WOODI*.

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Since the discovery of the earlier stages of *Simulium neavei* Roub. in phoretic association with the fresh-water crab, *Potamonautes niloticus* (M.-Edw.) (McMahon. 1951), widespread surveys have been carried out in western Kenya in order to determine the extent of the disinfesting operations necessary. It soon became apparent that phoresis was occurring in rivers far from haunts of *S. neavei*. This was confusing, as adult flies were not captured in the vicinity of these rivers nor did the terrain appear to possess features essential to the existence of this species. These rivers flow through flat country and are generally devoid of bush, offering little or no shade, with shallow banks incapable of diverting even the lightest of breezes; humidity was generally low. As efforts to capture adults during normal feeding times were unsuccessful, intensive catches were instituted and were made in the early morning, midday and late evening but still with negative results. The earlier stages that were collected exhibited morphological characters almost identical with those of *S. neavei* and it was not until adult flies were bred out that it was possible to decide whether a new species had been discovered. The imagines proved to be remarkably similar to *S. neavei*, the only macroscopic differences being the presence of pale areas on the legs of both males and females, and the presence of golden hairs on the basal third of the hind tibiae of the male, as opposed to uniformly dark legs in both sexes and a patch of not more than six golden scales at the basal tips of the hind tibiae in the male *S. neavei*. A critical examination of the male genitalia, however, left no doubt that the material was distinct from *neavei* and in fact it proved to be of *S. nyasalandicum*, a species described by De Meillon (1930).

Further confusion was introduced when larvae and pupae were observed to be living in the exhalant passages of the branchial chamber of the crab (fig. 1). Larvae thus found are remarkably similar to those found externally, but the pupae exhibit minor differences which a superficial examination would overlook. The intrusion was at first deemed to be fortuitous, and it was thought that it was merely an extension of the habit exhibited by *S. neavei* when larvae and pupae are sometimes found in the eye socket. But it was noticed that larvae living in the exhalant passage were extremely reluctant to leave the seclusion afforded by this habitat and when removed at once made attempts to return to it. A closer examination of the pupae showed that specific differences were discernible. Differences peculiar to the specimens frequenting the exhalant passages as opposed to those found on the external surfaces of the crab. When bred out, the imagines were found to be of *S. woodi*, which has morphological characters distinct from both *S. neavei* and *S. nyasalandicum*. It would appear that both associations, external as well as internal, are obligatory, as the earlier stages are never found except in association with the crab. Superficially, all three species are remarkably similar and might easily be confused one with the other, more especially *S. neavei* with *S. nyasalandicum* as these two species

have many external features in common, as well as identical phoretic habits. But certain points of difference exist in all stages. The larvae closely resemble each other in nearly all respects, except that the menta possess distinctive characters; the lateral teeth vary in number and configuration and there are disparities in the number and arrangement of the spines on the lateral borders

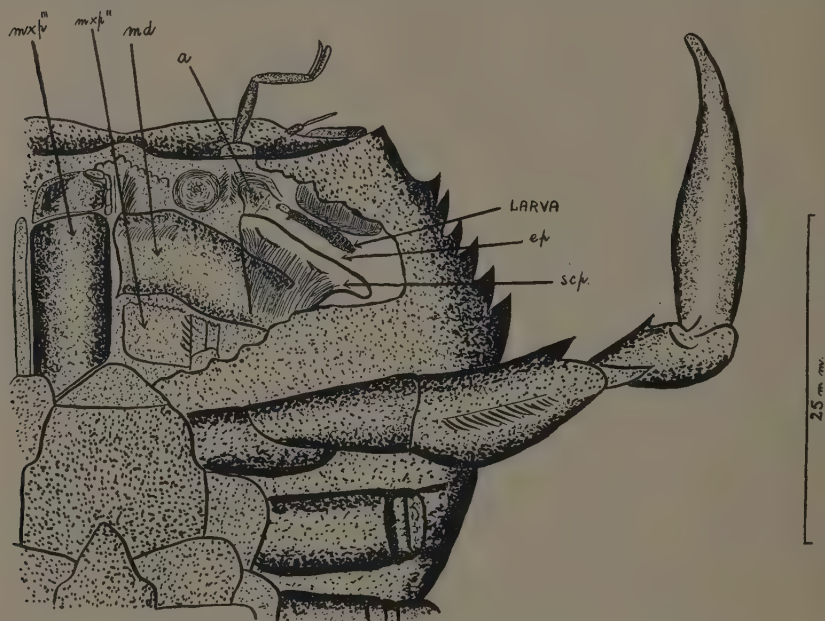


Fig. 1.—Diagram of ventral surface of *Potamonautes niloticus*, showing larva of *Simulium woodi* in position. Part of the sternum has been cut away to show the mouth-parts of the crab and the exhalant passage. a, aperture of exhalant passage; ep, exhalant passage; md, mandible; mxpli, 2nd maxilliped (part of); mxplii, 3rd maxilliped; scph, scaphognathite.

(fig. 5). Whereas the pupa of *S. neavei* and the pupa of *S. nyasalandicum* are identical, that of *S. woodi* has longer breathing filaments and the secondary and tertiary bifurcations occur at greater distances from the base (fig. 7). The cocoons of *S. neavei* and *S. nyasalandicum* are similar in texture and colour, being composed of fine threads, light brown in colour, and woven in a symmetrical pattern. That of *S. woodi*, on the other hand, is of coarser texture, much darker in colour, and of irregular pattern (fig. 6).

The adults are similar in appearance, more especially those of *S. nyasalandicum* and *S. neavei*. Certain characters exist, however, which enable them to be separated. The absence of a pronounced tooth on the tarsal claws of the female of *S. neavei* readily separates it from the other two species (fig. 2). Both sexes of *S. nyasalandicum* usually have pale areas on the tibiae and femora as opposed to uniformly black or dark brown legs in *S. neavei* and *S. woodi*. In some districts, however, *S. nyasalandicum* has been observed to possess black legs and if it were not for the toothed tarsal claw in the female, and the presence of pale hairs on the middle and hind tibiae of the male, identification would be

extremely difficult. *S. woodi* possesses features which readily separate it from the other two species; the scales on the thorax, abdomen and legs are pale and brassy in appearance as compared with golden scales in *S. nyasalandicum* and *S. neavei*. The long fringe of hair on the distal border of the scutellum is entirely black or with at least a black basal fringe. A conspicuous feature peculiar to

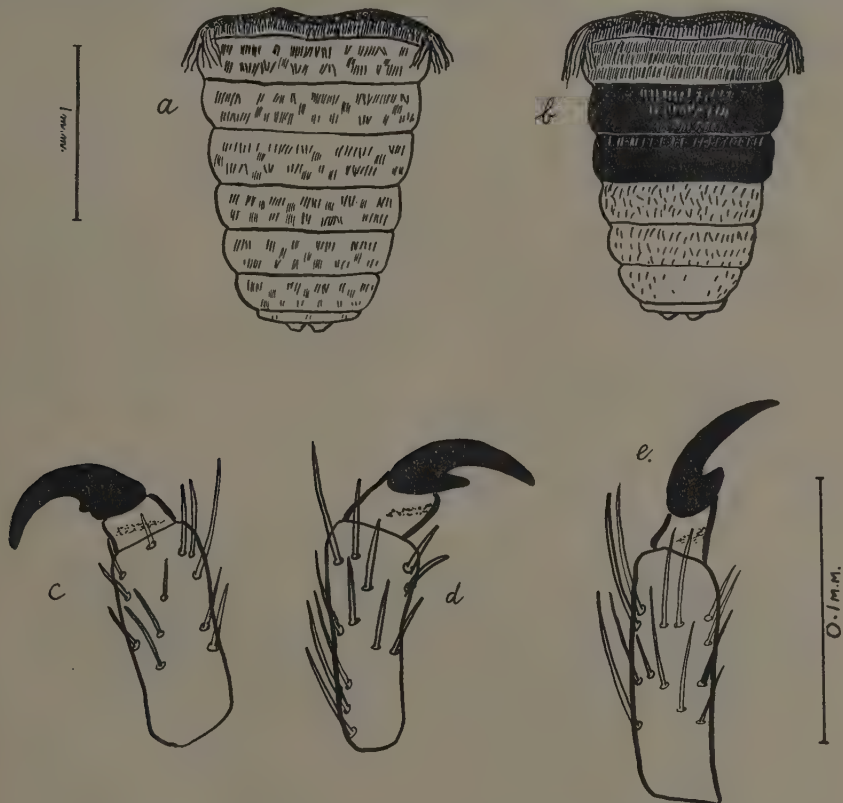


Fig. 2 (a, b).—Pattern on abdomen (partly diagrammatic) of female: a, *Simulium nyasalandicum*; b, *S. woodi*. (Note: The abdomen of female of *S. neavei* is similar to that of *S. nyasalandicum*.)
(c, d, e).—Fifth tarsus and claw of: c, *S. neavei*; d, *S. nyasalandicum*; e, *S. woodi*.

S. woodi is that the almost complete lack of pale hairs on the 3rd and 4th tergites in the female creates the impression of a conspicuous dark, transverse band (fig. 2). The male genitalia also exhibit distinctive features (figs. 3 & 4). The ventral plates differ in size and proportions, that of *S. woodi*, for instance, being larger than that of *S. neavei* and the U-shaped emargination is more pronounced in the former. The ventral plate in *S. nyasalandicum*, apart from being proportionately longer and narrower, has an extremely narrow and deep-cleft emargination which balloons out on the dorsal surface to form a pronounced elevated ridge; it also possesses a feature not seen in the other two, namely a tuberculoid protuberance between the basal arms. The coxites and claspers of *S. neavei*

and *S. woodi* are similar to each other but those of *S. nyasalandicum* are narrower and the clasper is smaller in proportion, in relation to its coxite (fig. 4).

Descriptions of the male and female forms of *S. neavei*, *S. nyasalandicum* and *S. woodi* are given in Freeman & De Meillon (1953) but for convenience the morphological differences between the three species, referred to above, are shown for comparison in figs. 2, 3 and 4. The immature stages of *nyasalandicum* and *woodi* are, however, described below with notes on their distribution and habits. The male and immature stages of *S. neavei* were described by McMahon (1951).

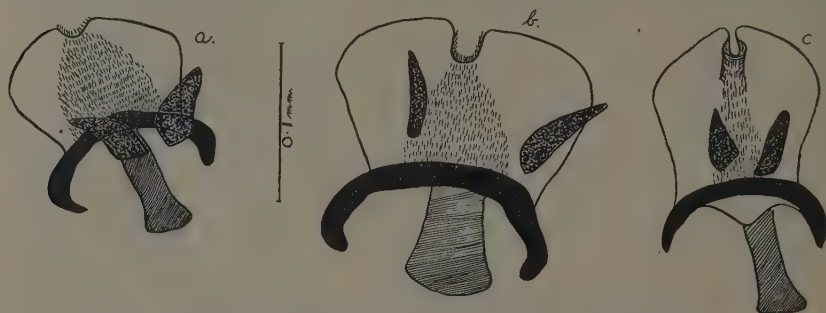


Fig. 3.—Ventral plates of: a, *S. neavei*; b, *S. woodi*; c, *S. nyasalandicum*.
All drawn to same scale.

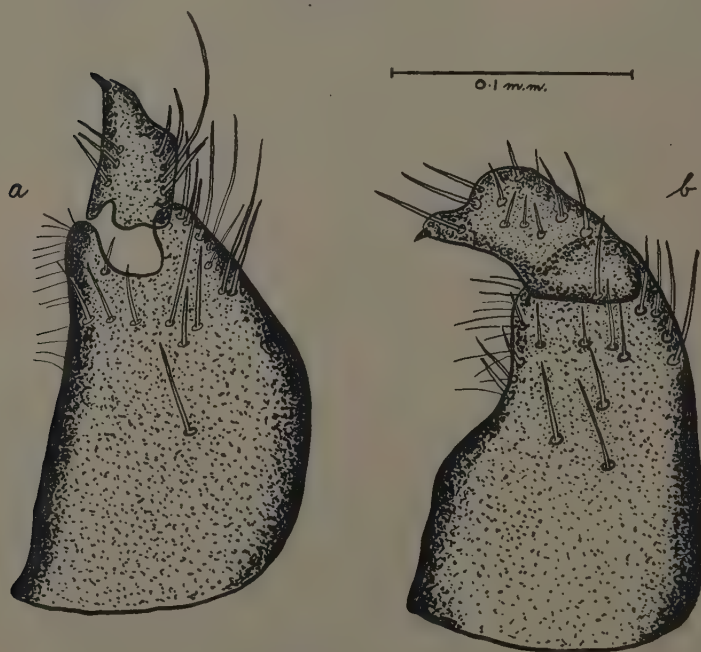


Fig. 4.—Coxite and clasper of: a, *S. nyasalandicum*; b, *S. neavei*.

Simulium nyasalandicum* De Meillon.Larva.*

Length 7 mm. General colour reddish brown closely approximating that of the carapace of the host.

Head.—Without pigmented areas. Antenna normal. Mandible (fig. 5, d) with a long main and two shorter strongly chitinised teeth; 6–9 secondary teeth protrude from the concave surface below main tooth. Mentum (fig. 5, b) with a terminal row of thirteen strongly chitinised teeth, the inner rather pointed and the outer two pairs larger and blunter; there are four to six small lateral teeth, confined to the distal third, larger and less sharply pointed than those of *S. neavei*; parallel with and just inside the lateral edge lie a row of 6–7 stout spines, quite often arranged asymmetrically; 15–26 short spines of varying length are to be seen in the medial area. Feeding brushes with approximately 45 long bristles as in *S. neavei*.

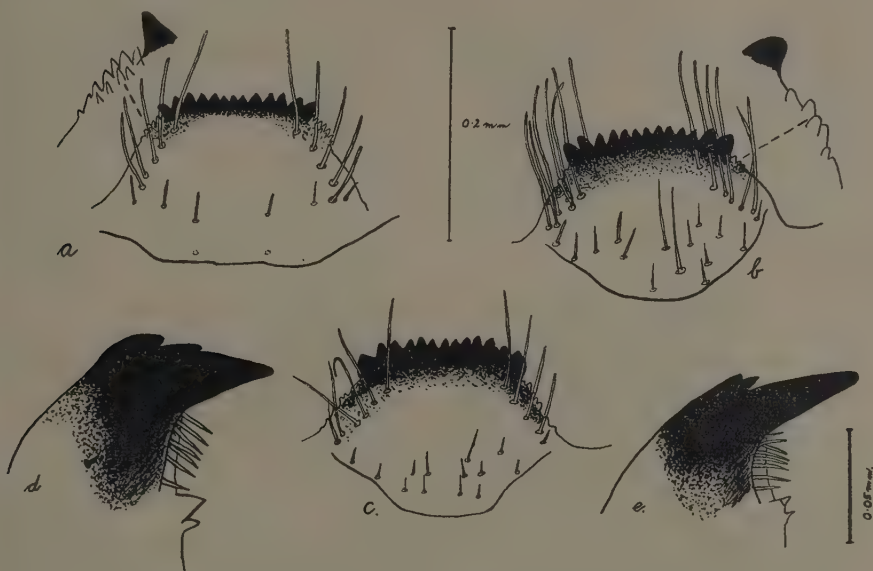


Fig. 5 (a, b, c).—Larval mentum of: a, *S. neavei*; b, *S. nyasalandicum*; c, *S. woodi*.
(d, e).—Lateral view of mandible of: d, *S. nyasalandicum*; e, *S. woodi*.

Thorax.—Pseudopod cylindrical, three times as long as broad and tapering distally.

Abdomen.—No features of taxonomic importance are exhibited.

Pupa.

Length 3.3 mm.

Head and thorax.—Smooth and not covered with disc-like tubercles; trichomes small, slender and simple. Respiratory organ (fig. 7, c) pale, slender and very long (3.74 mm.); the outer walls are covered with minute pigmented nodules not arranged in rows and the base is covered with disc-like tubercles. The filaments arise from three short main stems dividing into three pairs; two of the filaments again divide at a point less than 0.8 mm. from the base, making

eight in all. The respiratory organ is identical with that of *S. neavei* and approximates that of *S. hirsutum* Pomeroy, but the latter is much smaller and there is a difference in ratio of long and short filaments (5 long and 3 short in *S. nyasalandicum* as compared with 3 long and 5 short in *S. hirsutum*).

Abdomen.—Terminal segments with a pair of downward-projecting hooks. Dorso-lateral surface; 2nd, 3rd and 4th tergites with 14 hooks displaced as follows: two parallel rows of 4 strong, single hooks each side of the medial line and two rows of three small simple hooks near the lateral borders; the eight medial hooks on the 2nd tergite are about half the size of those on the 3rd and 4th. Tergites 5–7 are devoid of hooks but have from 12–16 spines arranged transversely. The eighth has two transverse rows of 10–14 downward-projected teeth on each side of the medial line; this is not a constant feature and is quite often absent. Ventro-lateral surface; third sternite with 1–2 small simple hooks each side of the medial line, but these are frequently absent. Fourth and fifth with four bifid hooks arranged in two pairs, one each side of the medial line; great variation, however, takes place and they are sometimes represented by two single and two bifid, three bifid and one single, four single or only two bifid. Sixth and seventh sternites with four hooks, two bifid and two single, the latter near the lateral border.

Cocoon.

Composed of pale slender fibres woven into a neat symmetrical pattern (fig. 6, b).

Note.—There are no discernible differences between the pupa of this species and that of *S. neavei*. They differ, however, from *S. woodi*, which is described later.

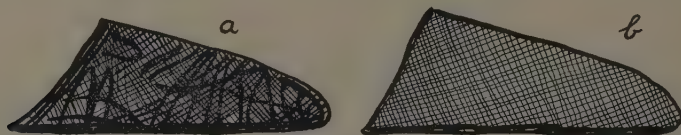


Fig. 6.—Cocoon of: a, *S. woodi*; b, *S. nyasalandicum*. (Note: The cocoon of *S. neavei* is similar to that of *S. nyasalandicum*.)

Distribution and habits.

S. nyasalandicum has a wide distribution and the indications are that it will be found wherever its associate, *P. niloticus*, occurs providing there is sufficient oxygenation. In Kenya it has been collected at 4,000 ft. in rivers entering Lake Victoria from the east, and at 5,900 ft., west of Eldoret. It has been found in all sorts of rivers, in open sunlit situations and in dense forest, but it seems to prefer the former conditions. Like *S. neavei*, it is rarely found in rivers and streams lacking cascades and waterfalls, even when the crabs are present in large numbers; this may be directly due to lack of oxygen or to the absence of essential foods caused by the same deficiency.

Adults are rarely captured on the wing although a number were caught in the upper reaches of the R. Nzoia, in an area of European settlement where large-scale farming is practised and where little bush exists. Although it is occasionally caught feeding on humans, it is thought that its food preference is for some other host, but this is difficult to ascertain as only very hungry flies come to feed and, consequently, previous blood meals cannot be detected by serological or other methods. The rôle it plays in connection with the spread of onchocerciasis is not known, as none of the very few flies caught were dissected. Should it prove to be a vector, then the elimination of this disease will be vastly complicated in

so far that a great deal of work will be entailed, both in the field and the laboratory, breeding out specimens in order to identify them with any degree of certainty. The indications are, however, that it is not a vector, otherwise people living in areas where only *S. nyasalandicum* is found would be suffering from this disease and this does not appear to be the case.

The females of both species have long been known. *S. nyasalandicum* was first collected by S. A. Neave on Mount Mlanje, Nyasaland, in 1914 and *S. woodi* by R. C. Wood at Cholo, Nyasaland, in 1917. They were given the status of distinct species by De Meillon in 1930 when he examined females from a collection in the British Museum (De Meillon, 1930).

***Simulium woodi* De Meillon.**

Larva.

Length 8.0 mm. General colour reddish brown.

Head.—Without pigmented areas. Antenna normal. Mandible (fig. 5, e) with a long main and two shorter chitinated teeth; 6-8 secondary teeth protrude from the concave surface below main tooth. Mentum (fig. 5, c) with a terminal row of 13 strongly chitinated teeth, the inner rather pointed and the outer three on each side rather larger and blunter. There are 7-8 small lateral teeth, irregular in shape and size, extending along the lateral border for two-thirds of its length, unlike those of *S. neavei* and *S. nyasalandicum* which are closely set and confined to the distal third. Parallel with, and just inside, the lateral border lie an oblique row of 4-6 stout, rather long, spines; 14-15 short spines are to be seen in the median areas. Feeding brushes with 45 long bristles.

Thorax.—Pseudopod long and narrow, tapering distally, about twice as long as width at base.

Abdomen.—No features of taxonomic importance are exhibited.

Pupa.

Length 3.5 mm.

Head and thorax.—Smooth and not covered with disc-like tubercles; trichomes small, slender and simple. Respiratory organ (fig. 7, a & b) dark, slender and very long (5.0 mm.); the outer walls are covered with minute pigmented nodules, arranged in rows, and the base is covered with disc-like tubercles. The filaments arise from three, rather long, main stems, each approximately 1 mm. in length; two of the filaments again divide at a point approximately 2 mm. from the base, making eight in all. The respiratory organ is similar to those of *S. neavei* and *S. nyasalandicum* but is much longer (5.0 mm. as compared with 3.74 mm.) and the basal stems are at least twice as long; secondary bifurcation takes place at a much greater distance (2 mm. as compared with 0.5 mm.).

Abdomen.—The abdominal armature is practically identical with that of *S. nyasalandicum* except that the two rows of 4 teeth on the second tergite are as large and strong as those on the third and fourth tergites and the downward-projecting teeth on the eighth tergite are invariably present, although quite often in smaller numbers. The pattern on the ventro-lateral surface is identical with those of *S. nyasalandicum* and *S. neavei* and is subject to the same variations on the fourth and fifth sternites.

Cocoon.

The cocoon (fig. 6, a) is an untidy gelatinous structure with coarse dark fibres irregularly interwoven in a background of lighter-coloured and more delicate fibres, as compared with those of *S. neavei* and *S. nyasalandicum*, which have regular neat patterns composed exclusively of pale slender fibres (fig. 6, b).

Distribution and habits.

The unique association of this species with *P. niloticus* was first observed on 8.xi.1950 during a routine survey carried out in the R. Sasala when many crabs were collected, nearly all of them infested with larvae and pupae. A larva was seen in what was at first thought to be the buccal cavity of a crab, but which eventually proved to be the exhalant passage leading from the gill chamber. Further investigations revealed that this is a common occurrence and many crabs were subsequently seen to have larvae or pupae existing in one or both passages. On the following day, searches in the R. Lusumu revealed that internal infestations occurred here also and that an even higher percentage of crabs was found to have larvae and pupae in these situations. As many as three larvae were found in one single passage and, in another crab, a fully grown larva and a pupa caused complete obstruction; great embarrassment would no doubt be experienced should both passages be simultaneously blocked as it would appear that the host does not possess a mechanism capable of ejecting its uninvited and, presumably, unwelcome guest. It is not difficult to imagine the reason for this intrusion; free board and lodging as well as complete immunity from all the usual hazards besetting young insects in fast-flowing water is indeed an achievement. It is not known, however, whether the larvae actually share the crab's food or whether this is extracted from the water pumped through the passage by the action of the scaphognathite; the former seems probable and, therefore, this association would appear to be essentially a commensal one.

The method of ingress practised by young larvae is not known but it seems improbable that they enter through the gill slits situated in front of the chela

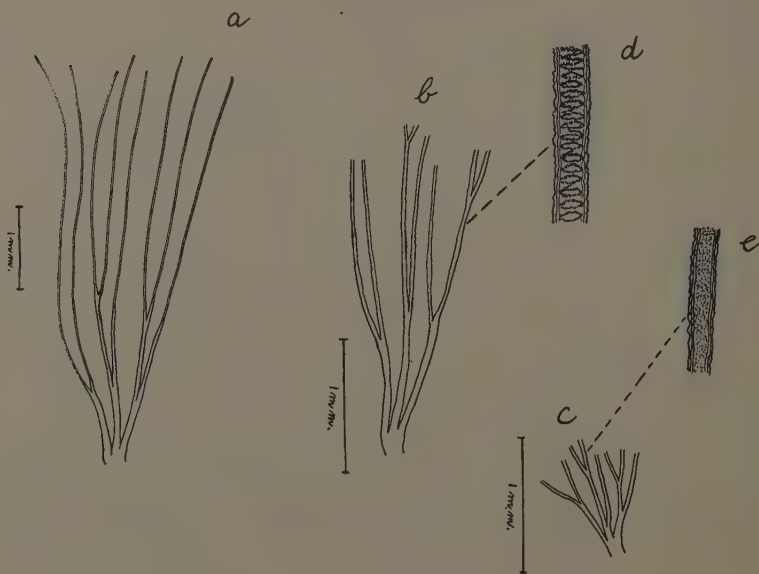


Fig. 7.—Respiratory organ of: a, *S. woodi*; b, the same, magnified to show greater lengths of main and secondary stems as compared with those of *S. nyasalandicum* (c); d, part of the same, further magnified to show pattern in *S. woodi* as compared with that of *S. nyasalandicum* (e). (Note: The breathing filaments of *S. neavei* are identical with those of *S. nyasalandicum*.)

and above the legs. The openings above the legs are long and narrow and young larvae might be crushed by the articular movements of the joints. The opening in front of the chela is larger but can be closed at the will of the crab. It would seem probable, therefore, that a direct entry is made into the exhalant passage through the large anterior opening below, and protected by, the tip of the third maxilliped.

Once the larva has reached its haven no attempt is made to leave it. Pupation takes place *in situ* and the adult emerges either below the water level or during one of the not infrequent visits paid by the crab to dry land. It is certain that this way of life is peculiar to this species because, of the many thousands of specimens removed from these situations, not one belonged to another species. It cannot apparently exist elsewhere as it has never been found on the external surface of the crab and it would appear, therefore, that it is vitally necessary for its existence to seek this very exclusive retreat.

The distribution of this species is far wider than that of *S. neavei* and it is to be found, like *S. nyasalandicum*, wherever its host occurs, always provided there is sufficient aeration. It seems to prefer, however, open sunlit situations to forests. The food preferences of the adults are not known, but it is certain that these do not include human blood as adults of this species have never been captured attempting to feed on man. In actual fact, over a period of some 12 years, not one single specimen has been captured on the wing during the course of many hundreds of catches made in Kenya under a variety of conditions. Consequently, nothing is known of its biology except that it does not play a part in the spread of human onchocerciasis, and, although of outstanding academic interest, it appears to be of no economic importance. It might, of course, be a vector of veterinary importance but wild adults have never been captured and, therefore, none have been dissected; it is thus not possible to make observations on this point.

It must obviously be considered a member of the *S. neavei* group in view of its similar pupal characters and its predilection for the same host, which it quite often shares with one or the other species, or both. The male genitalia, too, although larger in size and exhibiting divergent features, is of the same pattern. But it would appear from some of the adult characters that this species is less closely related than are the other two members of this group, whose almost identical appearance causes some confusion.

Summary.

Simuliid larvae and pupae found in phoretic association with the fresh-water crab, *Potamonautus niloticus* (M.-Edw.) in Kenya rivers far from the haunts of *Simulium neavei* Roub. gave rise to adults which proved to be *S. nyasalandicum* De Meillon, a member of the *neavei* group. Very similar larvae, and pupae that exhibited minor differences from those of *S. nyasalandicum*, were found in the exhalant passages from the gill chamber of the crab. These yielded adults of *S. woodi* De Meillon, and no other species was found in this particular situation; nor were examples of *S. woodi* found on the external surface of the crab. As many as three larvae were found in one passage and, in another crab, a fully grown larva and pupa caused complete obstruction of the passage on one side. Apart from the security enjoyed by the larva in the sheltered position, it is thought probable that the association is essentially a commensal one.

The morphological differences between the adults of the three members of the *neavei* group (*S. neavei*, *S. nyasalandicum* and *S. woodi*) are given and figured. The immature stages of *S. nyasalandicum* and *S. woodi* are also described and morphological differences are figured.

Notes are given on the distribution and habits of the two latter species, for

larvae, pupae and

neither of which is man the preferred host. In fact, over a period of some 12 years, not a single specimen of *S. woodi* has been captured on the wing during the course of many hundreds of catches made in Kenya under a variety of conditions.

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PRELIMINARY INVESTIGATIONS ON THE BIOLOGY AND
ECOLOGY OF THE PARASITES AND PREDATORS
OF *BREVICORYNE BRASSICAE* (L.).

By K. S. GEORGE

There are many records of the primary parasites of certain species of Aphids, the bionomics and development of some of which have been described by Gatenby (1919), Haviland (1920), and Vevai (1942). Spencer (1926) records *Diaeretus rapae* (Curt.) as a parasite of both the Cabbage Aphid, *Brevicoryne brassicae* (L.), and the Green Peach Aphid, *Myzus persicae* (Sulz.), while Petherbridge & Mellor (1936) record *D. rapae* as attacking *B. brassicae* in England and discuss, in general terms, the influence of parasites and predators upon this Aphid. The effectiveness of non-specific parasites and the whole problem of parasite specificity has been discussed in detail by Marcovitch (1935) who describes experiments on strip-cropping in America as a means of increasing the number of available enemies of Aphids. Ripper (1944) discusses biological control as a supplement to chemical control of *B. brassicae* and is alone in making a numerical estimate of the extent of parasitism in the field. Taxonomic work on the identification of the APHIDIINAE has been published by Marshall (1899) and Smith (1944).

The primary parasites of Aphids are restricted to two groups, the Braconid sub-family, APHIDIINAE, a cosmopolitan family containing the most important parasites, and the Chalcidoid family, APHELINIDAE. In this investigation, *D. rapae* (APHIDIINAE) was the only primary parasite recorded from the Cabbage Aphid. The secondary or hyperparasites obtained were identified as *Charips* sp. (CHARIPIDAE), *Asaphes vulgaris* Wlk. (PTEROMALIDAE) and *Lygocerus* sp. (CERAPHRONIDAE).

Field Technique, 1953 and 1954.

Samples were taken from parts of plants within experimental plots set up in two fields of brussels sprouts, one near Ixworth in Suffolk and the other near Maulden in Bedfordshire. Both fields were close to synoptic weather stations. Fortnightly sampling from May to October gave results which indicated the magnitude of fluctuations in density of Aphids, parasites and predatory insects within the crop.

On each field, the plot consisted of five adjacent rows of 40 plants which were numbered 1 to 200. At each fortnightly visit two separate samples, A and B, were taken and consisted of three leaves (one upper, one middle and one lower) from each of 50 plants (Church & Strickland, 1954). The leaves from the three categories were kept separately in waxed bags. Sampling throughout the season was so arranged that every plant was visited once in four weeks. Thus, in period 1, leaves were taken from plants 1, 5, 9, etc., for sample A, and from plants 2, 6, 10, etc., for sample B. On the next visit, plants 3, 7, 11, etc., and 4, 8, 12, etc., were sampled. At the third visit, plants 1, 5, 9, etc., were visited for sample B and so on. The samples were taken back to the laboratory and treated as follows:—

Sample A.—Each of the three sub-samples of upper, middle and lower leaves was taken separately and the Aphids washed off, leaf by leaf. In 1953, when there was a large number of Aphids on the samples, a washing machine was used; in 1954 a jet of water from a small hose-pipe was sufficient. The Aphids

TABLE I.

Total Aphids, mummies (total less initial number) and percentage parasitism of *B. brassicae* on both sites in both years.

Site and year	Date	Parts of plant										Total	
		Upper leaves		Middle leaves		Lower leaves						Aphids	Mummies
		Aphids	Mummies	%	Aphids	Mummies	%	Aphids	Mummies	%	Aphids		
Ixworth 1953	7 July	1558	0	0	56	0	0	55	0	0	1669	0	0
	21 July	1668	1	0.1	580	6	0.1	780	1	0.1	8308	8	0.1
	4 Aug.	768	34	4.4	3908	80	2.1	4380	33	0.8	9056	147	1.6
	18 Aug.	396	413	—	26316	891	3.4	15456	227	1.5	42168	1531	3.6
	1 Sept.	24	93	—	26176	1354	5.4	38796	450	1.2	63996	1897	3.0
Ixworth 1954	27 July	1128	12	1.1	2616	28	10.7	1620	14	0.9	5364	54	1.0
	10 Aug.	2836	0	0	15	1	6.7	12	1	8.3	2863	2	0.1
	25 Aug.	4	0	0	8	0	0	0	0	0	12	0	0
	7 Sept.	6	0	0	13	0	0	26	0	0	45	0	0
	21 Sept.	4	0	0	11	0	0	0	0	0	15	0	0
Maulden 1953	14 July	1	4	—	4	0	0	0	0	0	5	4	80.0
	28 July	49	0	—	1776	0	0	293	2	0.7	2118	2	0.1
	11 Aug.	276	29	10.5	3168	14	0.4	3456	18	0.5	6900	61	0.9
	25 Aug.	408	39	9.6	3180	252	7.9	1224	45	3.7	4812	336	7.0
	8 Sept.	444	25	5.6	12480	524	4.2	9552	134	1.4	22476	638	3.0
	23 Sept.	464	31	6.7	25548	904	3.5	16830	251	1.5	42842	1186	2.8
	7 Oct.	5	13	—	3312	413	12.5	12888	161	1.2	16205	587	3.6
	21 Oct.	0	5	—	810	272	3.4	4164	81	1.9	4974	358	7.2
Maulden 1954	3 Aug.	0	1	—	12	0	0	72	4	5.6	84	5	6.0
	17 Aug.	228	0	0	174	0	0	708	1	0.1	1110	1	0.1
	30 Aug.	50	0	0	72	0	0	242	2	0.8	364	2	0.6
	13 Sept.	78	8	10.3	546	5	0.9	1452	15	1.0	2076	28	1.4
	27 Sept.	1188	0	0	139	2	1.4	1560	26	1.7	2887	28	1.0
	11 Oct.	61	12	19.7	1548	36	2.3	1416	28	2.0	3025	76	2.5
	26 Oct.	641	1	0.2	552	24	4.3	1968	13	0.7	3161	38	1.2
	9 Nov.	62	2	3.2	41	1	2.4	648	4	0.6	751	7	0.9

The numbers of Aphids were derived from Samples A, of the mummies from Samples B.

were collected on fine gauze and transferred to tubes containing 95 to 98 per cent. alcohol. After a short time the mummies and the cast skins of the Aphids in the sample floated to the surface and these were decanted off. The Aphids were then counted, using an aphid counting grid (Strickland, 1954).

In 1953, alatae and apterae were recorded separately; in 1954, a further distinction was made between adult apterae and nymphs.

Sample B.—Each of the three sub-samples was examined and the visibly parasitised Aphids were removed into labelled specimen tubes. The leaves were then kept in insect-proof containers in an insectary and examined daily, and any parasite mummies formed during the preceding 24 hours were removed with a stiff brush into labelled tubes giving a succession of daily collections. In addition, predators or their eggs were removed as they were found and recorded separately. Fresh cabbage leaves were placed on the floor of each container at intervals so that Aphids falling from leaves had a suitable medium on which to live. The routine examinations were followed for 14 days from the date of collection, by which time there were very few, if any, fresh mummies to record.

Each day the insects which had emerged from the collected mummies were removed, identified, sexed and recorded. Frequent examination is essential during the peak emergence period because of the hyperparasitic habits of some of the insects involved. The examination of tubes was continued until the end of November, by which time there was no further insect emergence. The tubes of mummies were kept in the insectary throughout the winter and were examined periodically in the early months of the following year. A few insects emerged in March and April, and there was a peak of emergence in May, June and July.

Check sampling was carried out frequently. On each occasion two samples were taken from a block of 200 plants selected at random in the field. The Aphids on each of the two samples were washed off, counted and recorded. Pairs of figures thus obtained ranged from six and 74 on the one hand to 68,400 and 75,800 on the other. The figures indicate that the numbers of Aphids taken in the two samples are approximately the same when fairly high populations are being considered. The method of sampling, whereby the leaves from one set of plants are inspected for Aphids on one occasion and for parasite mummies on the next visit, ensures that no unnecessary bias is included in the counts.

The method described for assessing parasitism was time-consuming and a simplified method was designed to see whether a smaller sample would give similar results. The short method consisted of sub-sampling sample A before the Aphids were actually washed off. From each of the upper, middle and lower categories of leaves, 100 large nymphs and adults were taken at random and put on to fresh cabbage leaves (replaced by fresh leaves every three or four days) in gauze-covered trays. These Aphids were inspected daily and all mummies removed and recorded (Table VI). This method was compared with the main method in 1953. In 1954, there were so few Aphids in the main sample that the short method had to be discontinued.

Parasitism in the Field.

The relationships between parasites and Aphids, assessed at two-weekly intervals throughout the season, are given in Table I. The figures are based on aphid numbers and the total minus the initial numbers of mummies found in the samples. The mummies actually present on the plant at the time of sampling have been discounted because of the cumulative effect of mummies containing overwintering parasites. The proportion of mummies falling into this category was as high as 80 per cent. at the time of peak aphid infestation—in 1953 this was on 1st September at Ixworth after which the field and, unfortunately, the plots were sprayed by the farmer, and on 23rd September at Maulden. The apparent

increase in parasitism after the peak at Maulden was probably due more to a decline in the number of Aphids on account of climatic factors than to an increase in the number of mummies produced.

Parasitism in the upper leaf samples in 1953 was remarkably high (Table II), and may be explained on biological grounds: on the upper leaves, colonies tend to be small and diffuse, especially after the first few weeks of the "season". An ovipositing parasite does not have to make a prolonged search for Aphids as they are sufficiently numerous, and therefore a large proportion is parasitised. On the middle and lower leaves, where the aphid colonies are more dense, the parasites tend to restrict oviposition to those individuals at the edge of a colony because the wax and honeydew present quickly immobilise the parasites. A more critical point is that the Aphids in large colonies increase at such a rate that the parasites available cannot attack more than a small percentage of them: the proportion of parasites to Aphids can be seen in Table I.

In 1954, rainfall was high and temperatures low at the beginning of the season, and early migrations of Aphids failed to become established at Ixworth. Numbers fell to almost zero and parasites were completely absent. At Maulden the early colonies survived, but there was little increase in numbers.

Weather records were obtained in both years from synoptic stations adjacent to the sampling sites and such factors as rainfall, accumulated day-degrees and maximum and minimum temperature appear to be unrelated to fluctuations in aphid or parasite density.

Figures for overall parasitism on both sites during both years are presented in Table II.

TABLE II.

Overall percentage parasitism of *B. brassicae* in 1953 and 1954 in relation to total Aphids collected from the leaf samples.

Leaves		1953	1954
Upper	Overall percentage parasitism	11.3	0.6
	Total Aphids collected	6061	6286
Middle	Overall percentage parasitism	4.1	1.7
	Total Aphids collected	111594	5747
Lower	Overall percentage parasitism	1.3	1.1
	Total Aphids collected	107874	9724

At the end of the season in both years there was a considerable number of mummies from which the parasites had not emerged. The proportion of parasites overwintering in this way increased as the aphid season progressed (Table III).

From a high proportion of the overwintering mummies, no emergence took place, but where it did, emergence occurred from April to July of the following year with a peak in June-July. As the parasites and hyperparasites emerged from the mummies, they were identified, sexed and recorded. The proportions of individual insect species are recorded in Table IV.

In both years, the proportions of primary parasites exceeded those of any of the hyperparasites. At Maulden, in both years, the number of *Charips* sp. produced was far higher than the corresponding figures for Ixworth. Similarly, the proportion of Chalcidoidea and Proctotrupeoidea which emerged in 1954 was

far higher than in the preceding year. The number of these insects which emerged in the spring is correspondingly far higher than the number produced in the summer and in the field this may possibly be explained by the greater length of time that the mummies are exposed to attack. Parasitised Aphids from the samples were kept in tubes, and oviposition by hyperparasites must have

TABLE III.
Overwintering of parasites of *B. brassicae*.

Date	1953			Date	1954		
	Total mummies	Non-emergence	Percentage non-emergence		Total mummies	Non-emergence	Percentage non-emergence
July 7-14	5	0	0.0	July 27-Aug. 3	98	30	30.6
July 21-28	11	4	36.4	Aug. 10-17	44	29	65.9
Aug. 4-11	236	79	33.5	Aug. 25-30	45	19	42.2
Aug. 18-25	2231	833	37.3	Sept. 7-13	92	41	44.6
Sept. 1-8	4242	1595	37.6	Sept. 21-27	282	180	63.8
Sept. 23	3213	1623	50.5	Oct. 11	114	97	85.1
Oct. 7	1449	1344	92.8	Oct. 26	107	100	93.5
Oct. 21	1577	1551	98.4	Nov. 9	254	250	98.4
	12964	7029			1036	746	

The total mummies includes those on the leaves at the time of sampling which were not included in the figures given in Table I.

taken place in the field between the time of the death of the Aphid and subsequent collection of the mummies. In the field, the percentage of hyperparasites of this group is probably higher than that recorded from the samples. In the spring, the hyperparasites probably disperse in search of other, earlier-occurring hosts, for they are known to breed through several species of APHIDIINAE.

TABLE IV.
Proportion of parasites and hyperparasites of *B. brassicae* reared from material collected in 1953 and 1954.

		Summer emergence	% of total	Spring emergence	% of total	Total percentage
<i>Diaeretus rapae</i>	1953	5177	39.9	687	5.3	45.2
	1954	200	19.3	153	14.8	34.1
<i>Charips</i> sp.	1953	691	5.3	892	6.9	12.2
	1954	53	5.6	135	13.0	18.6
<i>Asaphes vulgaris</i> and <i>Lygocerus</i> sp.	1953	65	0.5	407	3.1	3.6
	1954	32	3.1	159	15.3	18.4
Non-emergence	1953			5041	38.9	40.0
	1954			299	28.9	28.9
Total emergence	1953	5933	45.7	1986	15.3	61.0
	1954	290	28.0	447	43.1	71.1

The percentage of mummies from which no insects emerged is high in all cases. A sample of the mummies collected in 1953 was examined in the spring of 1955, but none was viable. In the field, it seems unlikely that mummies remain viable after the first year, and the overall loss due to non-emergence and other factors is probably higher than that indicated by the samples.

In addition, the proportion of males and females of *D. rapae* which emerged were analysed to see whether the ratio differed significantly from the expected (say) 1:1. The percentage of females obtained is shown in Table V.

TABLE V.

Ratio of females to total of *D. rapae* obtained from samples.

Date	1953			Date	1954		
	Total	Females	Females in total (%)		Total	Females	Females in total (%)
July 7-14	1	0	0	July 27-Aug. 3	62	36	58.1
July 21-28	7	3	42.9	Aug. 10-17	9	4	44.4
Aug. 4-11	154	85	55.2	Aug. 25-30	9	2	22.2
Aug. 18-25	1360	857	63.0	Sept. 7-13	31	23	74.2
Sept. 1-8	2337	1476	63.2	Sept. 23-27	67	41	61.2
Sept. 23	1373	982	71.5	Oct. 11	34	21	61.8
Oct. 7	314	239	76.1	Oct. 26	59	35	59.3
Oct. 21	318	261	82.1	Nov. 9	82	42	51.2

In most cases in 1953 there is a significantly higher proportion of females than males; in 1954, the number of parasites produced was too small for significance to be apparent, but the figures are somewhat similar to those shown for 1953.

The results from the sub-sample taken from the 1953 samples, described on p. 621, are presented in Table VI. In a few cases, it was impossible to take 300 Aphids from each of the two sites because of the small size of the main sample.

TABLE VI.

Percentage parasitism in the 1953 sub-sample.

Date	Aphids	Mummies	Parasitism (%)
July 7-14	183	1	0.5
July 21-28	600	3	0.5
Aug. 4-11	600	23	3.8
Aug. 18-25	570	74	13.0
Sept. 1-8	550	146	26.6
Sept. 23	300	110	36.7
Oct. 7	280	102	36.4
Oct. 21	222	67	30.2

The trend of these results is for parasitism to increase until a peak is reached when the aphid infestation is at its highest. The figure then decreases slightly. The trend is similar to that obtained with the routine samples, the difference being in the magnitude of the results. About 30 per cent. of each sample consisted of adult Aphids whereas counts on field populations of *B. brassicae* in

1954 showed that, in fact, the actual ratio is very close to 14 nymphs to one adult, or about 7 per cent. throughout the season. The adults taken had been exposed to possible parasitisation for a longer period of time than the nymphs, and the increased parasitism is probably due to bias.

Predation in the Field.

Predatory Syrphid and Cecidomyiid larvae were taken in association with colonies of *B. brassicae* in the course of the field work (Table VII) and adult Coccinellids were frequently noticed but were rarely found actually on the aphid host-plants. Coccinellid larvae were noticed feeding on *Aphis fabae* Scop. on fat hen (*Chenopodium album*) growing in sprout fields but not attacking *B. brassicae*. On two occasions a small ectoparasitic red mite was seen on adults of *B. brassicae* but the Aphids did not die and the mites fell off and disappeared.

Syrphid larvae appear to be the most efficient predators of *B. brassicae* and the following species were recorded from the samples:—*Syrphus balteatus* (Deg.), *S. luniger* Mg. and *S. ribesii* (L.), the majority being *S. balteatus*, of which eggs, larvae and pupae were taken. The white, elongated eggs are laid in small groups or singly, normally on the underside of leaves and frequently on clean leaves, though they were not found on plants where Aphids were absent. Experiments in the laboratory showed that in the larval stages an average of 230 to 600 individuals of *B. brassicae* were consumed. The Aphids consumed were taken from colonies on sprout leaves from the fields.

TABLE VII.

B. brassicae—Predators collected from the samples taken in 1953 and 1954.

1953 Date	Total Aphids	Syrphids	Cecido- myiids	1954 Date	Total Aphids	Syrphids	Cecido- myiids
July 7-14	1674	37	0	July 27-Aug. 3	5448	28	0
July 21-28	10426	214	0	Aug. 10-17	3973	21	0
Aug. 4-11	15956	357	355	Aug. 25-30	376	18	0
Aug. 18-25	46980	267	313	Sept. 7-13	2121	102	17
Sept. 1-8	86472	238	717	Sept. 21-27	2902	35	19
Sept. 23	42842	151	1111	Oct. 11	3025	4	0
Oct. 7	16205	60	225	Oct. 26	3161	2	0
Oct. 21	4974	25	72	Nov. 9	751	0	0

During 1953, three parasite species emerged from Syrphid pupae:—*Diplazon laetatorius* (F.), *D. tarsatorius* (Panz.) and *Promethes dorsalis* (Hlmgr.). Only six specimens were obtained from about 150 pupae and no detailed observations were made on the abundance, habits and life-histories of these insects.

Cecidomyiid larvae (*Phaenobremia* sp.) were found only at Maulden, in both years. The very small, red, elongated eggs of these insects are laid in groups of up to 40 and on all occasions were found underneath existing colonies of *B. brassicae*. Experiments in the laboratory showed that between 40 and 60 Aphids are consumed by each larva. Careful search in several sprout fields showed that whereas these insects were quite common in the Maulden area, none at all were to be found in the Ixworth district.

Specificity of Primary Parasites of the Aphid.

Whereas a few parasites of Aphids are polyphagous or attack not more than about three species of Aphids, the majority is restricted to one host (Marshall, 1899). A population of primary parasites reared through one species of Aphid

will seldom attack another species even though it be close at hand and reaches its population peak at an optimum time.

The following field and laboratory experiments were designed to show the host range of *D. rapae*, the primary parasite of *B. brassicae*.

In the field, in 1953, observations were made on adjacent crops of field beans and brussels sprouts to determine whether the parasites of *Aphis fabae* on beans would attack *B. brassicae* on sprouts after the infestation of *A. fabae* had died out. Two acres of sprouts were set out with alternate rows of beans and these were visited regularly throughout the summer. Unfortunately, 1953 was a poor year for *A. fabae* and very few bean plants were infested. In consequence, parasitised Aphids were almost completely absent and the subsequent infestation of *B. brassicae* on the sprouts was not affected by the proximity of the bean crop.

On a smaller scale, plots were laid out at Cambridge consisting of adjacent areas of beans and sprouts but again the absence of *A. fabae* prevented any observations of parasite behaviour. Laboratory stocks of *A. fabae* were transferred to the bean plants but these failed to become established.

In addition to these two experiments, fields of sprouts adjacent to fields of beans were visited and collections made of parasitised material of *B. brassicae* on the three rows of sprouts nearest to the bean crop. The primary parasites which emerged from these mummies were identified as *D. rapae*.

In 1954, experiments were continued, observations being made on *B. brassicae*, *A. fabae*, *Myzus persicae* and *Aphis nasturtii* Kalt.

A plot was set up at Cambridge consisting of strips of potatoes, beans and sprouts arranged so that the margins of each crop were adjacent to each of the other two crops. During July, the beans became heavily infested with *A. fabae* and large numbers of the Aphids were parasitised. The parasites on the plot were carefully watched on many occasions and they rarely left the bean plants. When they did alight on the neighbouring sprouts and potato plants they moved about the leaves in their normal searching attitude but mostly left the plants within one minute. At this time of the year there were only a few small colonies of *B. brassicae* on the sprouts; contacts between these Aphids and the bean-aphid parasites were observed only on very few occasions and each time the parasite ignored the Aphid completely and either crawled away or flew to another leaf or to a bean plant. Collections of mummies of *A. fabae* from the beans were made at intervals until late September but in all cases where a primary parasite emerged it was identified as *Aphidius* (*Lysiphlebus*) *fabarum* Marshall. On two separate occasions a parasitised example of *B. brassicae* was found on a leaf of a bean plant. These two mummies were kept, and from one a female of *D. rapae* emerged, from the other, a female of *Charips* sp.

During August, all the sprouts had colonies of *B. brassicae* on them and there were also a few examples of *M. persicae* on some of the lower leaves; the potato plants had colonies of *M. persicae* and *A. nasturtii* on a few leaves only. The adults of *D. rapae* which emerged from the parasitised material of *B. brassicae* frequently came into contact with *M. persicae* on the same plant but they were never observed attacking this Aphid. The potato plants were frequently examined but the Aphids on them remained completely free from parasite attack.

Collections of parasitised Aphids were made in the field throughout 1953 and 1954. At Harlow, Essex, during August and September, parasitised material of *M. persicae* was collected from the leaves of cabbage plants. The primary parasites which emerged were all identified as *Aphidius matricariae* Hal. This site was the only one on which parasitised examples of *M. persicae* could be found. An adjacent field of sprouts was infested with *B. brassicae* and parasitised specimens taken from rows adjacent to the cabbage field produced only *D. rapae*.

Several fields of potatoes in the Bedfordshire area were visited and collections of parasitised material of *M. persicae* made from three fields adjacent to crops of sprouts produced *A. matricariae*.

At every opportunity, parasitised specimens of *B. brassicae* were taken from sprouts plants along the edges of fields and close to hedgerows, but *D. rapae* was the only primary parasite which emerged.

During the summers of 1952, 1953 and 1954, different species of Aphids and various parasites were put together in cages in the laboratory and the behaviour noted. In 1952, small cellophane cages were used but these suffered from condensation of moisture on the cage walls and this made observation of the insects difficult and trapped any parasites which settled on the surface. Bigger cages were designed and used in 1953 and 1954. These cages proved to be suitable for the experiments as the plants could be watered from below and parasites could be inserted by lifting one side of the cage for a few seconds. The Aphids used in the experiments were the progeny in each case of a few first-instar nymphs taken from colonies and then maintained on potted plants in insect-proof cages. Thus, completely parasite-free stocks were used in all the experiments.

The combinations of Aphids and parasites made and the results achieved are indicated in the appropriate column of Table VIII. A plus sign (+) indicates that oviposition took place and a minus sign (−) that there was no attempt at oviposition. The figures in brackets after the symbols show the number of times the experiments were repeated.

TABLE VIII.

Behaviour of three species of parasite in the presence of different species of Aphids.

	<i>Aphidius fabarum</i>	<i>Diaeretus rapae</i>	<i>Aphidius matricariae</i>
<i>A. fabae</i> on bean	+ (5)	− (3)	
<i>B. brassicae</i> on sprouts	− (3)	+ (8)	
<i>A. fabae</i> on bean and <i>B. brassicae</i> on sprouts } both in one cage	+ (3)	− (6)	
	− (3)	+ (6)	
<i>Macrosiphum rosae</i> (L.) on rose		− (4)	
<i>M. persicae</i> on cabbage		+ (5)	+ (3)
<i>M. persicae</i>		+ (6)	+ (3)
<i>B. brassicae</i> } on sprouts		+ (6)	− (3)
<i>M. persicae</i> & <i>B. brassicae</i> on sprouts and <i>M. persicae</i> & <i>A. nasturtii</i> on potatoes .. } in one cage		+ + (2)	
		− − (2)	

Observations were made on each of these combinations of Aphids and parasites for up to one hour daily. The observations were repeated during 1953 and 1954 and in many cases the combinations were replicated. When parasites were introduced into cages without their host-aphids most of the time was spent in crawling on the gauze or glass sides of the cages. When a parasite was introduced into a cage containing its host-aphid and another Aphid, the host-aphid was, in every case, thoroughly investigated first.

The case of *D. rapae*, introduced into a cage containing *B. brassicae* and *M. persicae* on a sprout plant is interesting. The parasite bred through the *M. persicae* as well as through its normal host-aphid, which was in marked contrast to the field observations already described.

Early experiments on the specificity of parasites of Aphids were made in small cages and in petri dishes. In a few cases *D. rapae* appeared to oviposit in *M. persicae* but the Aphids continued to live and were not in fact parasitised.

To determine whether the parasites actually oviposited on these occasions the following experiments were carried out. Several turnip seedlings were grown in Knops solution containing ^{32}P at concentrations of between 300 and 400 microcuries per litre. *B. brassicae* feeding on these plants became radioactive, giving counts after three days of between 700 and 1,000 per minute on a G.M. counter. Parasites bred through the Aphids gave counts of between 100 and 200 per minute. This was disappointingly low but the parasites were allowed to oviposit in adults of *B. brassicae* and the latter were then exposed to a Geiger counter and a scaling unit. There were no positive counts at all and the conclusion was that at this level of contamination by radio-phosphorus there is not sufficient deposited in the egg to be detectable.

Summary.

During 1953 and 1954, experimental plots were set up on two sites in eastern England and regular fortnightly samples were taken in an attempt to evaluate the degree of control of the Cabbage Aphid, *Brevicoryne brassicae* (L.), exerted by its parasites and predators.

The plot at each site consisted of a block of 5 rows of 40 plants and each main sample consisted of a sample of three leaves (one upper, one middle and one lower leaf) from 25 per cent. of the plants. Two such samples (A & B) were taken at each visit and were so arranged that the plants were sampled in strict rotation. The leaves were taken back to the laboratory and sample A was used to assess the number of Aphids, the other for an assessment of parasitism. The Aphids were removed from sample A and put into 95 per cent. alcohol and counted. The mummies were removed from sample B and put into labelled tubes. The leaves from sample B were then kept in insect-proof containers and examined daily for the next fortnight. All mummies which formed were removed and sorted. The insects which emerged from the mummies were killed, sexed and recorded. Predators taken from this sample were identified and recorded and used for further experiments. The validity of counting Aphids from one sample and parasites and predators from another was investigated by a series of "check-samples" taken from arbitrary plots in the fields. A shorter method for assessing parasitism was tried, using a sub-sample of the main sample, and an explanation is given for the disparity between the two sets of results.

The figures obtained from the samples showed that parasitism ranged from 0 to 7.2 per cent. and, at the peak aphid infestation, is approximately 3 per cent. Many of the mummies contain overwintering parasites and approximately 6 weeks after Aphids are first found on the crop, 0-60 per cent. of the parasites fail to emerge. By the end of the season this figure has risen to 95-98 per cent. Of the insects which emerged, *Diaeretus rapae* (Curt.), the primary parasite of *B. brassicae*, was most common. The hyperparasites, *Charips* sp., *Lygocerus* sp. and *Asaphes vulgaris* Wlk. varied in numbers from site to site and year to year. Of the primary parasites taken throughout the season, approximately 60 per cent. were females.

The predators taken were SYRPHIDAE, mainly *Syrphus balteatus* (Deg.), and CECIDOMYIDAE, *Phaenobremia* sp. Coccinellids were remarkable by their absence. In addition, the following Syrphid parasites were bred from collected larvae and pupae: *Diplazon laetatorius* (F.), *D. tarsatorius* (Panz.) and *Promethes dorsalis* (Hlmg.).

An investigation was started into the specificity of *Diaeretus rapae*. In the field it appears to attack only *B. brassicae*, but in the greenhouse it was induced to breed through *Myzus persicae* (Sulz.). Field and laboratory experiments were made using *B. brassicae*, *M. persicae* and *Aphis nasturtii* Kalt. with different primary parasites.

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TRIALS OF RESIDUAL INSECTICIDES AGAINST ANOPHELINES IN AFRICAN-TYPE HUTS.

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Davidson (1953) has published the results of a series of trials he made at Taveta, Kenya, using different preparations of the three chlorinated hydrocarbon insecticides, DDT, BHC and dieldrin, against *Anopheles gambiae* Giles and *A. funestus* Giles. After Davidson's departure in August 1951, the present author took over the experimental site and continued the investigation with the principal aims of evaluating the promising new material, dieldrin, of reconciling field and laboratory findings (*e.g.*, Davidson, 1953; Hadaway & Barlow, 1949, 1952) and investigating such matters of practical importance as the effect of repeated applications, smoke, etc. In this paper, frequent comparison is made with Davidson's results.

The experiments were brought to an abrupt end in August 1955 by the complete spraying of all houses in the area as part of the Taveta-Pare Malaria Control Scheme.

Experimental Details.

(i) Main experiments.

The experimental procedure followed was changed in only minor ways from that of Davidson (1953), both to make the two sets of observations comparable

and to avoid confusing the African staff. The procedure is given briefly in the following account, in which the points of divergence from the earlier routine are indicated.

Observations were made in trap huts of the type first used by Muirhead-Thomson (1950). These huts are 10-ft. square, constructed of native materials, light-tight except for a window, 1-ft. square, on one side, in which a lobster-pot type of exit trap is fitted. A double door was used, the outer mosquito-net door being closed when search was carried out in the hut. An African slept in each hut from 9.30 p.m. to dawn, and mosquitos attracted by this bait entered beneath the eaves. It was assumed that, when trying to leave, they made for the only source of direct light and entered the window trap. It was found most necessary to ensure that the hut was really light-tight and to reduce the space at the eaves to a narrow slit. If this was not done, it was found that, if a known number of mosquitos was released in a hut, a large proportion was not recovered.

Trials were first made in the ten trap huts at Msheksheni left by Davidson. These were completely re-roofed, the internal plaster removed and replaced by fresh, and all woodwork such as doors, window frames and beds scrubbed with coarse yellow soap. The floor was whitewashed. Pre-treatment catches showed that this process of renovation removed all sources of contamination and it was carried out whenever the treatment in a hut was changed.

Many trials were prolonged for a year or more, and to accommodate extra experiments another six huts were built to the same pattern about a mile away, near the large springs at Njoro Nkubwa. A small grass structure was made at the same site to house the traps and the mosquitos removed from them. The only change in the structure of the huts was the use of reeds (mixed *Phragmites* and *Typha*) for thatching at both sites, for reasons both of availability and extensive local utilisation of this material. In some later tests, huts were roofed and completely lined with dried banana leaf as examples of native types of construction with non-absorbent material. The mud used for plaster was dug on site and used either as it was found or mixed with an equal amount of river sand. The "pure" mud was sometimes reinforced with about 5 per cent. of short-fibred sisal waste, or cow-dung in rather greater proportions. Local practice is to use local soil unmixed for the initial plastering of new houses. It is an extraordinarily poor material for this purpose, and when large holes appear in the walls they are stopped, if at all, with a mixture of mud and dung.

Towards the end of the investigation, the soil from the two sites was tested by Dr. A. B. Hadaway and Mr. F. Barlow for sorption of insecticide, and I am indebted to them for the information that the grey soil at Msheksheni is very active but the slightly reddish one from Njoro Nkubwa is much less so. The red soil used by Davidson and obtained from a locality some miles from Taveta turns out to be deceptive, for it is lateritic but of relatively low activity (Barlow & Hadaway, 1955, Table VI). Whenever mention is made in this paper of "activity" or absorptive power of a substance or a surface (used in the sense of the external coating or layer of a wall), it refers exclusively to the removal of insecticide, in the vapour phase, from the external surface and its adsorption on the internal surfaces of the substance. Capillary absorptive power is distinguished by the use of such terms as "porous" or "pervious".

Huts have been treated with a variety of formulations, mostly wettable powders, using a knapsack pressure sprayer containing the calculated quantity of insecticide for treatment of the hut at the required dosage, with due allowance for waste. Sample papers were pinned on roof and walls for all treatments and from these the mean dosage of insecticide actually on the surface was estimated. All figures for DDT and dieldrin refer to amounts of technical DDT (80% 1,1,1-trichloro-2,2-di(p-chlorophenyl)ethane) and dieldrin (85% 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene) and those

for BHC to the γ isomer, which is assumed to be 12.5 per cent. of the technical BHC actually estimated. The suspension was made of sufficient volume to permit covering the internal surface twice. Thus there was no chance of leaving any part completely untreated due to running out of suspension; and the second coverage probably reduced the unevenness of the first one. The spray lance was always moved vertically at about 18 in. from the wall, except when a trial was given to the horizontal method advocated by some authorities. The latter was at once abandoned since it appeared to offer no advantage but several serious disadvantages. Until February 1954 the hollow-cone type of nozzle supplied with the pump was used. An investigation of some types of nozzle available (Burnett & Woodcock, 1956) demonstrated the inefficiency of this particular type and particularly the important influence of variations in pressure and in spraying distance in determining the proportion of the expended insecticide that reached and remained on the surface to be treated. A change was made to the more efficient fan type of nozzle. In the early trials, the importance of spraying distance and of pressure, except in determining flow rate, was not appreciated and although pressure was standardised very early, at 60 lb. per sq. in., a short lance was used, with consequent considerable variations in spraying distance, even during a single stroke, in many of the earlier treatments. This probably explains the considerable and unexpected variation in apparent dosage in these trials. In addition, the hollow-cone nozzle gives an inherent variation in deposit of 3 to 1 across its swathe, which is easily increased to 4.5 to 1 with a slight degree of overlap. Also, owing to pressure of work in the chemical section, the number of test papers was cut to a level that now appears to have been too low; and their positioning, although standardised, was probably not the best that could have been devised. In particular, some were placed too near corners and the pitch of the roof, where, owing to the shape of the spray cone, they may have received a double spraying. Thus the estimates of the deposits in some of the early treatments (this applies in particular to the DDT trials) were probably far too high. On average, both in Davidson's and the present work it was found that, with the hollow-cone nozzle, about 50 per cent. of the applied insecticide was wasted, and the investigation of nozzle characteristics showed that a loss of at least 35 per cent. is more or less unavoidable under the most favourable conditions using the equipment, pressure and spraying distance chosen. Since all treatments were made with the same allowance for waste, it is likely that the true variation in deposit from trial to trial was less than that shown by the chemical estimates. With the change to the fan nozzle, wastage was cut by a half, to about 30 per cent. of the total expenditure, inherent variations due to pressure and distance were much reduced, and a more even swathe was sprayed. Thus, more confidence may be placed in the figures given for the deposits in the later trials. Before this, the use of a 4-ft. lance, standardised pressure, special care in mixing the suspension and particularly an increased number of better-placed test papers had already improved the situation considerably. Thus nearly all the dieldrin and BHC deposits are accurately known. The better suspensibility of the BHC powder, compared with that of DDT or dieldrin, always gave more consistent mean recoveries with much less variation from paper to paper. All applications were made by the author.

One departure from Davidson's spraying procedure was made. Except in a few early trials, the floor was covered, when spraying, to forestall complications due to insecticidal dust which might be disturbed therefrom. Tests were made to see if this could have been an important factor in earlier results. When only the wall of a hut was to be treated, the roof, door and window frame were protected. If only the roof was to be sprayed, the walls were covered.

The same series of catches was made as Davidson had carried out—morning and evening window traps, and floor catches twice daily on five days per week.

Hand catches were discontinued early. Davidson had reduced these to one only per week, on Fridays, and it was found that these catches included very few mosquitos. If caught, these insects would have been removed after less than their natural period of contact with the insecticide. Catches were not made at week-ends, so it was assumed that these insects, instead of being caught on Friday, would either leave on Friday night or die. They would be swept out on Sunday afternoon before the evening window trap was put up, but their place, so far as the experiment was concerned, had been taken by insects which entered before dawn on Sunday. Thus there seemed no point in making these catches except for information on resting sites, etc., which had already been obtained by Davidson, and they were discontinued. Mosquitos removed alive from the window traps were kept in cages supplied with sugar water until next morning, mortality counts being made as required.

Throughout this paper the word "mosquitos" refers to females of the two local vectors of malaria, *A. gambiae* and *A. funestus*.

(ii) *Tests for fumigant-type and contact actions.*

Tests were made fairly regularly for true vapour or for particulate action (Davidson & Burnett, 1953). About 20 female mosquitos (blood-fed) were placed in small cages. One cage was suspended in the middle of each hut at the level of the eaves, another was placed on two pegs driven into the wall at chest height. The side of the latter cage nearest to the wall was about $\frac{1}{4}$ in. from it. The cages were introduced at 7 p.m. and removed at 7 a.m. next morning and the mortality was taken at noon.

A number of devices were tried to enable a comparison to be made of the persistence of insecticide on walls and roof, but none was satisfactory until carbon dioxide was utilised as an anaesthetic, to facilitate handling of the test mosquitos. Small shallow wooden trays were filled with the plaster used on the wall of each house and similar boards were covered with the roofing material. A number of these panels were suspended in appropriate positions, prior to the treatment of a hut, and sprayed with insecticide at the same time and in the same way as the hut. They were virtually part of the wall or roof. At intervals, panels were removed for tests. For these, twenty *Anophelines* were anaesthetised with carbon dioxide and tipped into a glass filter funnel, the stem of which was plugged with cotton-wool. The appropriate panel was inverted, any loose particles being allowed to fall, and was then placed over the funnel, which was attached to it with rubber bands. The panel was stood on edge. As the mosquitos revived, they settled on the test surface. At the end of half an hour (extended as surfaces became less lethal) carbon dioxide was introduced through the funnel stem and the anaesthetised mosquitos tipped into a cage. Mortality was taken about 20 hours later. In some experiments which were started before the development of this technique, and in others when the wall samples had disintegrated, the funnel was held against the wall by a retort stand. It was very difficult to find areas sufficiently flat for this procedure. Of course no distinction can be made in these tests between direct contact and the action of vapour released from the test surface. After tests the panels were returned to the huts.

(iii) *Contamination.*

Davidson experienced little trouble with contamination, but, in the early part of 1952, contamination of the mosquitos caught in the untreated huts (hereinafter referred to as the control catches) became serious. All traps and cages were tested each week by introducing mosquitos, and contamination was found in every month in which deaths in the control catches rose above 10 per cent., except January 1955 (Table II). Even when no contamination of traps or cages was

detectable by test, high, rapid mortalities among the control catches might show its presence. In these cases it may have been due sometimes to contamination of the tubes used to handle the insects, even though these were rinsed in cleaning spirit, but it is more likely to have been due to insecticide introduced into the hut by the human bait, either on his skin, clothes or blankets. Every endeavour was made to prevent loans of blankets or visits to other huts, but some may have taken place.

Contamination was finally checked in the middle of 1952 by a combination of frequent tests, rigid segregation from any possible source of contamination of apparatus used in and catches taken from the untreated huts, the replacement of decontamination with solvents by thorough washing with hot water and coarse soap, and the collapse of the laboratory under a falling tree, which caused a move to another shelter. There were sporadic cases of contamination after this, but they were not serious and were overcome with a minimum of delay; their occurrence is indicated in Table II. If contamination of the control catches took place it is obvious that catches in the treated huts were also affected. In this case it is less important, for many mosquitos affected by the insecticide in the hut will have died within it before they could be subjected to extra exposure in contaminated traps and cages. Further, all catches from treated huts have been corrected by Abbott's formula* (Abbott, 1925) for mortality in the control catches, which includes deaths due to contamination, presumably of the same order as that affecting the catches from the treated huts.

(iv) *Ants.*

Considerable variations have been noted in the total number of mosquitos caught in different huts in the same block. These were usually explicable on the basis of relative proximity to the source of mosquitos (*cf.* Wharton, 1951) or relative attractiveness of the bait. In the latter case, switching baits (with due regard to decontamination) tended to reverse the order of the catches. In late 1953, variations were found that were not readily explained as above, and moreover in huts where kills fell well below what was expected as a result of previous experience. Release of several hundred mosquitos within several different huts gave total recoveries, in the traps and on the floor, varying from 10 to 90 per cent. Since all apertures were sealed, low percentage recoveries were not likely to be due to escapes, and investigation showed the presence of a small, sandy-coloured ant, which was removing all dead mosquitos from the floor. These ants were so inconspicuous that it took several weeks to convince the staff that they were important. Bait mosquitos were put down in all huts, and it was found that only some were infested by ants. However, repeated baiting tended to attract the ants, which began regularly to invade huts that they had probably previously visited only on scouting trips. Baiting was therefore reduced to two nights per month. Cement troughs, filled with water to exclude ants (Davidson, 1953, Pl. V, fig. 3), existed around only some of the houses and were in any case ineffective since the insects could evade the trough by coming up in the mud and wattle walls. Attempts to keep them in check were made with poison bait and grease-bands, for direct application of insecticide could not of course be used, but nothing proved of much permanent value.

Infestation tended to increase during the dry season and diminish in the rains, and became progressively worse in 1954 and 1955. On examining past records it was found that some peculiar results in the past had been obtained in one or two particular huts that were now found to be badly infested. These trials are indicated later. Regular baiting made it possible to detect infestation in current trials, and this is shown in the relevant Tables. The results of these trials are

* This formula is given, in a slightly different form, by Fisher & Yates (1953, p. 12).

not entirely valueless; the effect of ants is always to produce an apparent mortality below the true figure, since they remove, almost exclusively, the dead mosquitos from the floor, and were never found in the traps. The treatments most affected are those that kill quickly, with a high proportion of insects dying in the hut, *i.e.*, BHC treatments. In the early stages the effect on percentage mortality recorded is negligible, since the insects in the traps also die, but later, when many of these live, but a high proportion of the total catch is still found on the floor, the removal of the latter would have the effect of greatly reducing the recorded mortality. DDT and dieltrin are less affected, since in the former case few mosquitos are found dead in the huts (see, for example, the Tables in Davidson, 1953) and in the latter case death is slow, and many of the mosquitos that are taken in the traps die even when the experiment has been going some months. Thus the proportion of the total dead insects lost is smaller than is the case with BHC.

TABLE I.
Monthly rainfall at Taveta Sisal Estate.

Month				Year			
				1951	1952	1953	1954
July	in.	0.64	0.57	0.59	0.12
	days		1	1	1
Aug.	in.	0.0	.05	0.39	0.39
	days		1	1	4
Sept.	in.02	0.19	0.24	0.13
	days		1	1	1
Oct.	in.	2.92	0.16	0.93	1.98
	days		2	6	4
Nov.	in.	5.63	1.87	5.64	2.57
	days		4	11	7
Dec.	in.	3.32	1.65	0.77	2.34
	days		4	6	4
				1952	1953	1954	1955
Jan.	in.	0.82	0	0.69	0.14
	days	2	0	5	1
Feb.	in.	2.52	.07	0.34	3.15
	days	5	1	2	6
Mar.	in.	2.72	4.38	1.81	2.60
	days	9	4	3	5
April	in.	4.27	4.34	7.86	0.95
	days	10	9	11	3
May	in.	2.59	3.24	4.17	3.34
	days	5	9	8	8
June	in.	0.0	0.58	0.07	1.0
	days	0	2	1	3
Total for Season	in.		days	25.45	17.20	23.50	18.71
				—	38	56	47

(v) Meteorological observations.

The numbers of mosquitos caught in the huts varied greatly with the season and with the year and the proportion of the two vector species also varied, the numbers of *A. gambiae* being relatively greater during the rains. Further, rain, or relative humidity, has been found to have a considerable effect on mortality (see p. 661). The rainfall for the period, by months, is given in Table I. I am indebted to the management of the Taveta Sisal Estate Ltd. for these figures, measured at their offices about 1 mile from Msheksheni and $1\frac{1}{2}$ miles from Njoro Nkubwa. No other meteorological records were regularly made.

Results.*(i) Mortality in the control catches.*

It was not always possible to carry out experiments in batches, since observations on many treatments carried on so long that many of the huts would have been out of use at any given time. Deaths in the control catches varied from time to time and it is necessary to compensate for this, and so all mortalities have been corrected by Abbott's formula (Abbott, 1925). The mean monthly mortalities in the control catches for the whole period of the experiments are given in Table II. Since all mortalities in catches from treated huts are quoted for 4-week periods from the date of treatment, and all references to "months" refer to 28-day periods, deaths in control catches for identical periods have been calculated and applied appropriately. Table II is given to show the seasonal

TABLE II.

Deaths among female mosquitos captured in control huts.
Mean mortality per cent. presented by calendar months.

Month	Year				
	1951	1952	1953	1954	1955
Jan.	—	11 (12)*	2 (2)	6 (6)	13 (20)
Feb.	—	8 (9)*	9 (9)*	6 (6)*	2 (3)
Mar.	—	8 (11)*	4 (6)*	3 (4)	6 (16)*
April	—	10 (14)*	2 (3)	4 (4)	3 (6)
May	—	7 (9)*	12 (18)*	3 (3)	1 (1)
June	—	12 (12)*	6 (7)*	4 (4)	1 (1)
July	—	4 (5)	5 (7)	1 (9)	1 (1)
Aug.	—	13 (23)*	1 (1)	2 (6)	0 (0)
Sept.	—	7 (9)	3 (5)	2 (7)	—
Oct.	—	12 (13)*	4 (5)	4 (7)	—
Nov.	—	3 (3)	3 (3)	6 (6)	—
Dec.	13 (13)*	1 (1)	5 (6)	3 (5)	—

The first figure in each entry applies to DDT, BHC and aldrin experiments, the second to dieldrin treatments (see text, p. 638). Asterisks indicate months during which some contamination was detected.

variation only. Two figures are given for each month. Davidson kept the mosquitos from his evening window traps until next morning, when the mortality was noted, about 14 hours after removal of the traps, whereas the morning-trap mortalities were recorded after seven hours. If he kept the morning catches until next day, mortality tended to increase more rapidly in the catches from the control than from the treated huts. As a check on this finding, two control mortalities were calculated, one using morning-catch mortalities seven hours, the other 26 hours, after removal of the traps from the huts. For both calculations

the same evening-catch mortality (14 hours after removal) and floor catch were used. The two control mortalities so obtained were used to correct the corresponding observed mortalities. In the case of dieldrin trials the later mortality so corrected was always as great as or greater than the earlier one and the effect of these treatments could be fairly assessed only by using the 26-hour mortality. With other insecticides the corrected mortality sometimes appeared to decrease with time, as in Davidson's experiments. For insecticides other than dieldrin, therefore, the 7-hour mortalities, corrected by the corresponding control mortalities, have been used. In Table II the first entry for each month gives the total control mortality when morning catches were kept for seven hours, the second entry is when they were kept for 26. It should be mentioned that the mortalities of Table II includes deaths suspected to be due to indemonstrable contamination, the true mortalities in the control catches probably being lower than those in the Table in some cases.

Because the experiments were not usually done in batches they are probably best classified under the insecticides used, apart from investigations of such topics as partial treatments and the augmenting effect of repeated applications.

Where relevant, certain of Davidson's results are included for comparison. These are taken from Macdonald & Davidson (1953) where they are corrected for control deaths. To facilitate cross references, all experiments have been re-numbered consecutively as they appear in the Tables, *i.e.*, the first entry in a Table bears a number one higher than the last entry in the previous Table.

When the total number of mosquitos captured in a hut in a four-weekly period was less than 20, no mortality has been calculated and the entry in the Tables has been marked accordingly.

(ii) *Treatments using DDT alone* (Table III & fig. 1).

All treatments have been made with the same preparation—Murphy "DeDeTane" dispersible powder (50% technical DDT). The trial of particular interest is no. 1. For reasons given earlier (p. 633) it is thought that the estimated dosage is high. The total recovery on the surface of the hut of 3.4 g./m.² is 82 per cent. of expenditure, a virtual impossibility with the apparatus used. Several test papers were damaged and so this deposit is the mean of nine only and the true deposit is probably closer to the 2.5 g./m.² used by Davidson (first line in Table) with which it is meant to be compared. If so, it will be seen that at these dosages the greater absorptive activity of the plaster used in trial 1

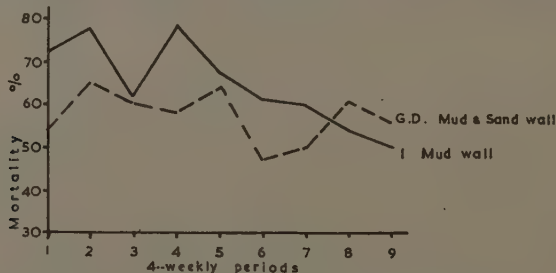


Fig. 1.—Percentage mortality among females of *A. gambiae* and *A. funestus* entering huts completely treated internally with DDT wettable powder. Trial 1 (Table III) is compared with the corresponding one made by G. Davidson (labelled G.D.). Mortalities are calculated for consecutive periods of four weeks from the date of spraying and corrected for deaths in control catches.

TABLE III.
Percentage mortality of mosquitoes in huts treated with DDT wettable powder.

Expt. Ref. No.	Type of test	Mean deposit of DDT (g./m. ²)	Type of wall	Date of spraying	Mortality in 4-weekly periods from date of spraying (corrected for mortality in controls)									
					1	2	3	4	5	6	7	8	9	
†G. Davidson	Whole hut	2.5	Red soil and gravel	Mar. 1951	54	65	60	58	64	47	50	61	56	
1	do.	3.4 roof 3.5 wall	Active mud	31/vii/52	72	77	62	78	67	61	60	54	50	
2	Wall only	1.8	Active mud and gravel	29/xi/51	50	49	28	—	—	—	—	—	—	
3	do.	3.1	Active mud	31/vii/52	52	45	27	37	19	—	—	—	—	
4	do.	0.9	Active mud	26/ii/53	30	23 (2 wks.)			—	—	—	—	—	
5	Roof only	2.7	—	8/iv/52	54	58	37	36	—	—	—	—	—	
6	do.	3.8	—	31/vii/52	50	52	40	—	—	—	—	—	—	
7	Floor only	1.6	(Concrete floor)	26/ii/53	40	20	—	—	—	—	—	—	—	

† Macdonald & Davidson (1953, Table II).

TABLE IV.
Percentage mortality of mosquitos in huts treated with BHC wettable powders.

Expt. Ref. No.	Type of test	Mean deposit of BHC (g./m. ²)	Type of wall	Date of spraying	Mortality in 4-weekly periods from date of spraying (corrected for mortality in controls)														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
†G. Davidson	Whole hut	0.23	Red soil and gravel	Mar. 1951	100	92	70	35	26	26	0	—	—	—	—	—	—	—	—
8	Whole hut	0.24 0.27 wall 0.2 roof	Active mud	31/vii/52	97	98	96	93	84	80	51	60	79 (3 weeks)	—	—	—	—	—	—
9	do.	0.27 0.23 wall 0.33 roof	Active mud	14/iv/53	100	97	66	75	34	45	58	—	—	—	—	—	—	—	—
10	do.	0.19 0.14 wall 0.26 roof	Active mud	18/iii/54	97	93	88	88	85	60* 70* 31* 19* 28	6*	—	—	—	—	—	—	—	—
11	do. subject to smoke	0.25 0.3 wall 0.18 roof	Active mud	26/ii/53	97	96	95	91	79	78	58	51	68	71	50	34	57	57	56
11A	do. retreated	0.21 0.18 wall 0.24 roof	do.	22/iv/54	100	93	85	56* 41* 13* 35* 63*	6* 44	34* 49	100* 63	—	—	—	—	—	—	—	—
12	Whole hut	0.26 0.24 wall 0.29 roof	Active mud and sand	11/vi/54	75* 73* 75* 70* 60	29	42	—	—	—	—	—	—	—	—	—	—	—	—
13	do.	0.23 0.22 wall 0.25 roof	Banana leaf	29/iv/53	96	92	65	51	21	—	—	—	—	—	—	—	—	—	—
14	Wall only	0.2	Active mud	14/iv/53	98* 73* 24* 5*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	Whole hut; lindane	0.14 0.12 wall 0.16 roof	Active mud and sand	18/iii/54	98* 95* 95* *† *†	}													
16	do.	0.15 0.14 wall 0.18 roof	Active mud	18/ii/54	99* 93* 88* *† *†														

† Macdonald & Davidson (1953, Table III).

* Depredations by ants demonstrated in this period

† Catches too small to calculate mortality.

has little effect in reducing the kill. At this period a satisfactory method of making contact tests had not been worked out, and it is not known if the walls were still lethal to the end. Trials 5 and 6 (see section *ix*) indicate that the roof alone would not give such a high kill and so presumably the wall was still contributing to the total kill. Particulate action persisted for at least three months, giving 100 per cent. kill.

The other treatments in Table III are discussed in a later section.

(iii) *Treatments using BHC alone* (Table IV & fig. 2).

These experiments suffered much from ants. Most of them were made using the Plant Protection formulation, "P520", a 50 per cent. wettable powder of technical BHC, at an intended rate of about 0.22 g. γ BHC per square metre. The most important finding was the prolonged action of this dose in a hut with a mud wall (Table IV, 8-11, & fig. 2). This contrasts with the result obtained

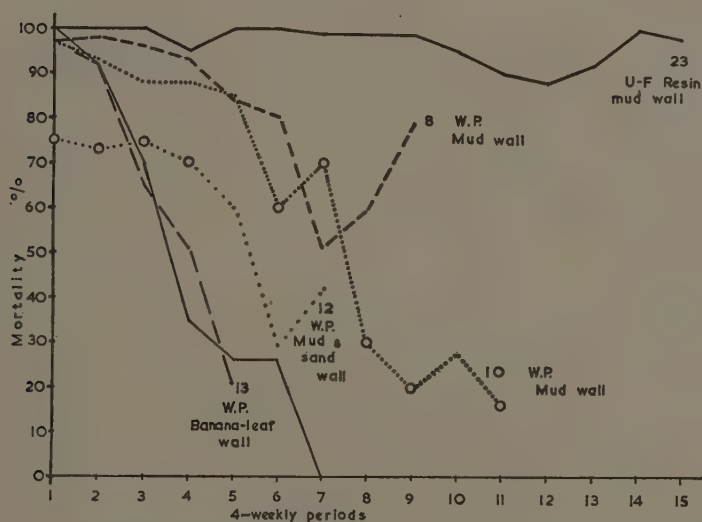


Fig. 2.—Percentage mortality among females of *A. gambiae* and *A. funestus* entering huts completely treated internally with BHC. Except for the top line, which represents BHC in urea-formaldehyde resin lacquer at 2.3 g. γ isomer/m.², all treatments were made with wettable powder at about 0.24 g. γ isomer/m.² (Tables VII & IV). Mortalities calculated as in fig. 1. Points shown by circles denote periods during which ants were detected in the hut. The number of each trial is indicated, and the result of one by G. Davidson (unlabelled solid line) is also included.

by Davidson in a hut plastered with inactive red soil and gravel, when there was a rapid falling off after three months (Table IV, line 1). This rapid loss from an inactive surface has been confirmed in a hut completely lined with impervious banana leaf (Table IV, 13, & fig. 2). The first treatment (8) of an absorbent surface (Table IV, 8, & fig. 2) was so striking in its effect that the trial was repeated (9). Unfortunately the hut chosen was one later found to be most persistently invaded by ants, and the previous finding was not adequately confirmed. Another attempt was made in February 1954 with partial success (10). The hut was invaded in the sixth month but even so the kill in the seventh month was 70 per cent., at a time of the year (August) when the seasonal decline

TABLE V.

Contact tests carried out in BHC-treated huts. Percentage mortality of fed female mosquitos.

Expt. Ref. No.	Surface	Four-weekly period during which test carried out														Period						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	21	22	23	24	25	26	
10	Wall Roof	—	—	100	—	100	—	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—
		—	—	0	—	47	—	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
11	Wall Roof	—	—	100	—	—	—	—	—	—	32	—	—	—	—	—	—	—	—	—	—	
		—	—	45	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	—	—	
11A	Wall Roof	—	—	100	—	100	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
		—	—	88	—	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
12	Wall Roof	100	—	100	31	42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
		100	—	78	12	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
15	Wall Roof	—	100	89	—	5	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
		—	19	14	—	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
16	Wall Roof	—	—	100	—	27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
		—	—	0	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
13	Wall Roof	—	100	—	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	(N.B. Banana leaf)	—	70	—	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
23	Wall Roof	100	—	—	100	—	100	0	100	—	100	—	—	—	—	—	—	—	—	—	—	
	(BHC in U; F. resin)	0	—	—	68	—	0	0	100	—	100	—	—	—	—	—	—	—	—	—	—	
																		</				

in mortality (see p. 659) would have its maximum effect. Further confirmation comes from another ant-infested hut (12) which was plastered with active mud and sand in an attempt to duplicate Davidson's experiment. At the time it was not known that his red soil was not highly active. Kills were never as high as is expected with BHC, owing to the ants, but even so did not fall below 60 per cent. until the sixth month (fig. 2). This suggests that the sand was not of great importance, since there was sufficient absorbent surface to retain insecticide, and that Davidson's rapid loss of effect was due to the slow absorption by his soil, permitting a large proportion of his insecticide to volatilise in the early stages.

Since contact tests showed the prolonged lethality of mud-plastered huts to reside principally in the wall (Table V) one hut was treated on the wall only (Table IV, 14). This was before ants had been detected but the hut was later found to be one of their favourites, and the results are therefore inconclusive. Trial 11 was part of an experiment to test the effect of wood smoke and tar which is described later. Mortalities run parallel to those of trial 8 remarkably closely (fig. 9).

Trials 15 and 16 (Table IV) were made to determine the persistence of lindane compared with deposits derived from technical BHC (trials 10 & 12). A 50 per cent. lindane wettable powder was used. Ants finally caused abandonment of the tests, but since very few mosquitos indeed were found in the window traps even after three months, it seems likely that high mortalities were still being maintained and that ants were removing the corpses from the floor. There is no indication that the lindane powder was less persistent than the technical BHC, or that the sand in the plaster had any marked effect. Both treatments had persisted longer than Davidson's when they were discontinued owing to low total catches.

Thus, in suitable conditions of ventilation and relative humidity, BHC in huts plastered with actively absorbent mud can give considerable kills for nine months after application, but it is of course unknown by how much this period would be reduced by increased ventilation in a dilapidated native-owned hut. On completely non-absorbent surfaces the lethal effect barely persists for twelve weeks. In huts with mixed plaster, provided the mud used is active, the persistence of BHC is greater than in huts completely built of non-absorbent materials.

(iv) Wettable powders of mixed DDT and BHC (Table VI & fig. 3).

Davidson (1953, p. 246) tried an oil-bound suspension containing DDT and BHC, with promising results (Table VI, line 1, & fig. 3). Since wettable powders are usual for application to mud walls, mixed DDT and BHC powders were tried. No commercial preparations being available, a hut was sprayed first with BHC (P520) and afterwards with DDT (DeDeTane). Separate test papers were exposed for each spraying and about 1.1 g./m.² of each chemical was intended, but only 0.7 g./m.² of technical DDT and 0.9 g./m.² technical (0.11 g. of γ) BHC were obtained. The results (Table VI, 17) were far less encouraging than Davidson's, although his preparation contained only one-quarter as much BHC as DDT and his actual deposit of BHC was lower. Another hut was treated with a modified wettable powder produced in the laboratory by adding to 50 per cent. DDT wettable powder (DeDeTane) enough γ and α BHC to simulate 20 per cent. technical BHC in the finished product. This was not of very good susceptibility, but sufficed, and the two insecticides were applied in the correct proportions but at rather over the intended dosage of 1.5 g./m.² of DDT, and 0.1 g./m.² of γ BHC (18). The results were encouraging, exhibiting the high initial kill of BHC, without the sudden deterioration found by Davidson (fig. 3). It was obviously necessary to obtain more information on the exact effect of

TABLE VI.
Percentage mortality of mosquitos in huts treated with mixtures of DDT and BHC.

Expt. Ref. No.	Type of test	Mean deposit of insecticide (g./m. ²) *	Type of wall	Date of spraying	Mortality in 4-weekly periods from date of spraying											
					1	2	3	4	5	6	7	8	9	10	11	12
†G. Davidson	Whole hut	2.5 DDT } (O.B.S.) 0.08 BHC }	Red soil and gravel	Mar. 1951	94	86	70	58	80	55	41	58	55	49	42	34
17	do.	0.7 DDT } (W.P.) 0.11 BHC }	Mud and gravel	25/xi/51	99	63	32	27	22	—	—	—	—	—	—	—
18	do.	2.1 DDT } (W.P.) 0.12 BHC }	do.	8/iv/52	99	96	96	86	85	82	73	68	78	81	70	52
19	do.	1.2 DDT } (W.P.) 0.15 BHC }	Mud	19/xi/52	77	55	76	45	56	50	44	40	34	34	—	—
20	do.	1.2 DDT } (W.P.) 0.1 BHC }	Mud and sand	19/xi/52	78	49	56	11	33	—	—	—	—	—	—	—
21	do.	0.9 DDT } (W.P.) 0.26 BHC }	Mud	19/xi/52	97	50	50	(2 wks.)		—	—	—	—	—	—	—
21A	do.	2.1 DDT } (W.P.) 0.19 BHC }	Retreatment	30/i/53	92	86	80	76	75	67	68	50	48	23	—	—
22	do.	1.3 DDT } (W.P.) 0.16 BHC }	Mud and sand	19/xi/52	82	67	29	66	35	(2 wks.)		—	—	—	—	—

† Macdonald & Davidson (1953, p. 804).

* BHC as γ isomer.

W.P. = Wettable powder.

O.B.S. = Oil-bound suspension.

dosage, and also substrate, and I am indebted to Mr. G. A. Emery for the supply of two samples of mixed wettable powders. These were compounded of 50 per cent. DDT wettable powder (DeDeTane) and enough lindane to produce a mixture containing, in terms of technical BHC, a quantity (a) equal to, and (b) half of, the amount of DDT, respectively. On subsequent analysis it was found that

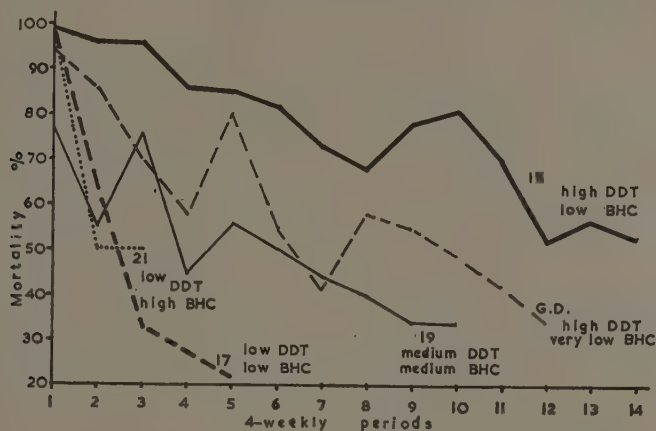


Fig. 3.—Percentage mortality among females of *A. gambiae* and *A. funestus* entering huts completely treated internally with mixtures of BHC and DDT; all were wettable powders except the oil-bound suspension used by G. Davidson (labelled G.D.). The exact dosage rates and the type of substrate are given in Table VI. The number of each trial is shown. Mortalities calculated as in fig. 1.

these amounts had been exceeded. The deposits of the two insecticides were estimated from the same test papers simultaneously but the BHC, being present as lindane, represented too small a proportion of the total deposit for satisfactory estimation, and the fact that the estimated deposits vary rather widely in some cases from what was intended may be due in part to this (Table VI, 19–22). One hut was treated twice. None of the treatments gave as good a kill as expected, the only one to approach treatment 18 in effectiveness being the retreatment of 21. It will be seen that all three trials which gave good results (that by G. Davidson, 18 and 21A) received over 2.0 g./m.² of DDT, although the amount of BHC varied from 0.08 to 0.19 g./m.² (γ isomer). It is realised now that the exceptional performance of trial 18 was due in part at least to the activity of the mud in the plaster used in this trial but not in the others, which retained sufficient BHC to maintain high kills for many months (fig. 3). It is now known that the soil used for the plaster in the later trials (19–22), at the Njoro site, is not very active, and that the red soil used by Davidson is also of low activity. In Davidson's trial, the relatively heavy deposit of DDT must have been important since this would by itself be capable of giving kills of over 50 per cent., after all the BHC had volatilised. It is also likely to have been important in trial 18, for 17 received almost as much BHC, but was ineffective (fig. 3). It is thought that the low dose of DDT in the latter case, whilst not sufficient to contribute to the total mortality, may have, in many cases, stimulated mosquitos to escape before they had picked up a lethal dose of BHC or mixed insecticides. By the end of the trials in this section it had been found that at lower cost BHC alone on absorbent walls, and dieldrin on non- or semi-absorbent plaster, exceeded the mixtures in effectiveness and the use of mixtures was not pursued further.

(v) *BHC in urea-formaldehyde resin* (Table VII & fig. 2).

A suggested use for insecticides formulated as urea-formaldehyde "blooming" resins has been to apply them on active substrates. Of the samples supplied, the only one to arrive undamaged was one containing BHC at the rate of 20 per cent. on the resin solids. This proved difficult to apply and was eventually diluted with solvent and applied with the usual knapsack sprayer. The mixture caused paroxysms of weeping, coughing and sneezing and the application was completed with face and eyes covered but does not seem to have been excessively poor. The mean deposit on the roof was 2.7 g./m.² and on the wall 2.3 g./m.². We are informed that this deposit is 90 per cent. γ isomer, therefore the equivalent deposits, as γ BHC, are 2.4 g./m.² on the wall and 2.1 g./m.² on the roof. This enormous dose gave consistently high kills for 15 months, after which mortalities fluctuated, although this was partly due to invasion by ants. This preparation is much more expensive than wettable powders and offers little prospect of use in African huts. It is unfortunate that preparations of non-volatile insecticides were not available in time for them to be tried, but it seems unlikely that they would be any better. Contact tests in the treated hut showed that the long-continued residual effect was localised largely in the walls of absorbent mud (Table V, last 2 lines). This confirms in the field what has been demonstrated in the laboratory by Barlow & Hadaway (1955), that absorbent plaster will remove insecticide from the skin of resin, although the process may be slower than with wettable powders. A non-volatile insecticide will become non-available in these circumstances and the resin lacquer will offer no advantage over wettable powders, or at least none comparable with the extra cost of manufacturing and applying the product. On impervious materials, BHC will be lost more rapidly than it was in this experiment and is therefore not likely to be of use as a resin.

(vi) *Aldrin* (Table VIII & fig. 6).

In the search for an insecticide that would give a prolonged kill on absorbent walls, a trial was made with aldrin. The sample available was an early one designed for agricultural use, of a reddish colour and not very readily creamed. It was applied at a mean rate of 1.1 g./m.². This is higher than the rate at which dieldrin was used but lower than the rate of application of BHC if this is expressed as the technical product which was actually used. (The figures for γ BHC should be multiplied by eight to give the deposit in these terms.) A high kill was obtained for two months, but mortalities in the third were negligible (Table VIII & fig. 6). Fumigant and contact tests all gave negative results in the third month and most of the insects from the window traps remained alive. This hut was later found to be ant-infested but there is no doubt that the insecticide was ineffective by the third month. The nearest approach to this treatment, with BHC, was at 1.52 g./m.² technical (0.19 γ isomer) shown in Table IV (10) which was far more persistent. Davidson used smaller amounts with conspicuous lack of success but these were in the region of 0.4-0.6 g./m.² (0.05-0.07 γ isomer) on walls containing gravel and are not a fair comparison (Davidson, 1953). It appeared that aldrin was less persistent than BHC, but it might be worth repeating the trial at higher doses. Cost for cost, however, BHC appears to be better.

(vii) *Diieldrin* (Table IX & figs. 4, 5 & 6).

Davidson made one trial with diieldrin (Table IX, line 1, & fig. 6). He was unable to have the deposit estimated and assumed, on the basis of his previous experience, that half the insecticide applied would be deposited, i.e., 50 mg./sq. ft. = 0.54 g./m.² (Davidson, 1953, p. 242), but given as 0.6 g./m.² in Macdonald & Davidson (1953). It was thought desirable to try a slightly lower dose, 0.45 g./m.², which was in fact the equivalent in cost, at that time, o

TABLE VII.

Mean monthly mortality per cent. of female mosquitos in a hut treated with BHC in urea-formaldehyde resin lacquer.

Expt. Ref. No.	Deposit of γ BHC (g./m. ²)	Type of wall	Date of spraying	Mortality in 4-weekly periods from date of spraying														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
23	2.4 wall	Active mud	29/iv/53	100	100	100	95	100	100	99	99	99	95	88	92	93	100	98
	2.1 roof																	
Expt. Ref. No.	Deposit of γ BHC (g./m. ²)	Type of wall	Date of spraying	Mortality in 4-weekly periods from date of spraying														
				16	17	18	19	20	21	22	23	24	25	26	27	28	29	
23	2.4 wall	Active mud	29/iv/53	65*	100*	74*	76*	61*	79	74*	74*	58	69	78	78	80*	40	
	2.1 roof																	

* Ants detected in hut during this period.

TABLE VIII.

Mean monthly mortality per cent. of female mosquitos in a hut treated with aldrin (40% wettable powder).

Expt. Ref. No.	Deposit (g./m. ²)	Type of wall	Date of spraying	Mortality in 4-weekly periods		
				1	2	3
24	1.1 wall 1.0 roof	Active mud	14/iv/53	99	95	26
						29
						4

TABLE IX.

Percentage mortalities of mosquitoes in huts treated with dieldrin formulations.

Expt. Ref. No.	Type of test	Formu- lation	Mean deposit (g. m. ²)	Type of wall	Date of spraying	Mortality in 4-weekly periods from date of spraying corrected for mortality in the controls																																						
						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30									
+G. David- son	Whole hut	25% W.P.	Expected to be 0.6	Red soil and gravel	March 1951	100	100	100	100	90	91	87	81	88	95	91	94	91	84	74	82	73	62	65	54	68	67	51	49	—	—	—	—	—										
25	do.	do.	Expected to be 0.45	Active mud and sand	28/xi/51	100	99	100	99	99	99	98	—	87	90	87	90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
26	do.	50% W.P.	0.49 0.5 wall 0.45 roof	Active mud	31/vii/52	100	100	96	89	75	64	44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
27	do.	25% W.P.	0.39 0.3 wall 0.52 roof	do.	30//53	99	90	89	91	70	66	49	59	43	50	77	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
27A	do.	do.	0.41 0.38 wall 0.44 roof	do.	5/xii/53	99	99	96	96	91	95	97*	91	96	80	62	65	†	35	83	75	91*	75*	80*	59*	60	—	—	—	—	—	—	—	—	—									
28	Whole hut	50% W.P.	0.3 0.23 wall 0.32 roof	Semi-active mud & sand	30/i/53	95	91	89	92	84	64	31	35	45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
29	do.	25% W.P.	0.53 wall 0.5 0.45 roof	Active mud and sand	29/iv/53	99	90	97	71	72	77	90	92	73	59	44	50* 74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—									
29A	do.	do.	0.35 0.31 wall 0.39 roof	do.	11/v/54	100	99	88	96	93	73	85	87	32* 86	77	92	95	84	82	43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
30	Whole hut	do.	0.42 0.32 wall 0.56 roof	Banana leaf	29/iv/53	99	99	97	71	86	91	93	86	82	53	58	45	59	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
30A	do.	do.	0.29 0.29 wall 0.23 roof	do.	22/iv/54	98	94* 79	68	71	69*	65	79	39	20* 46	6	48	46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
31	Whole hut	do.	0.83 0.58 wall 1.1 roof	Active mud and sand	29/iv/53	100	100	100	100	98	98	99	99	97	93	95	95	97	100	98	93	83	56* 60	69	76	†	62*	87	99	83	90	82	87	65	—									
32	do.	do.	0.9 0.76 wall 1.1 roof	Semi-active mud	5/xii/53	92	99	100	92	92	96	92	87	85* 70*	91	61	63* †	30*	29	59	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
33	do. subject to smoke	do.	0.86 1.0 wall 0.64 roof	Active mud	30//53	96	90	94	84	76	87	43	37	39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
34	Whole hut	15% emulsion	0.3 0.3 wall 0.43 0.63 roof	Banana leaf	12/ii/54	95	98	87	88	87* 73	51* 68*	0* 31*	35	53* 13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
35	Roof only	50% W.P.	0.31 roof	—	20/xi/52	99	47	39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
36	Concrete floor only	do.	0.13	—	21/xi/52	54	14	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—								
37	Mud floor only	25% W.P.	0.27	—	11/v/54	100	100	12	weekly mortalities																		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

† Macdonald & Davidson (1953, p. 805).

* Ants detected this period.

† Catches too small for calculation.

2.2 g./m.² of DDT. A water-soluble dye was included in the mixture to give at least an idea of the proportion of the spray which remained on the wall, but unfortunately the dye decomposed during extraction. The plaster contained 50 per cent. of sand, and very high kills were maintained for even longer than in Davidson's trial, and the trial was discontinued after a year (Table IX, 25). When estimation of dieldrin became possible, a hut was plastered with pure mud and sprayed at a known mean dosage of 0.49 g./m.² (26). The result was in strong contrast to the previous two experiments, for the kill fell below 50 per cent. within seven months. This result was so unexpected that the trial was repeated. A sample of 50 per cent. wettable powder had been used, and since Davidson had used 25 per cent. wettable powder, the second trial was made with a similar sample. The intended application rate was again 0.45 g./m.² but assessment gave only 0.39 g./m.². Nevertheless the two experiments were remarkably similar in result (Table IX, 27). The two powders were also tried in mud-and-sand plastered huts (28 & 29). The 50 per cent. powder (trial 28) gave a lower deposit than expected (0.3 g./m.²) and very poor persistence, comparable with its performance at a much higher rate on pure active mud (26). A much better application was made with the 25 per cent. powder (trial 29), in fact the trial approximated closely to what the original dieldrin experiment (25) had been intended to be,—0.5 g./m.², but with very different results, kills in the eleventh and twelfth months being only 44 and 50 per cent. The hut used for this trial was never found subsequently to be subject to infestation by ants and these cannot have been a complicating factor. The performance was better than the same powder on plain mud (27), but the measured dosage varies in the same sense (0.5 g./m.² versus 0.39 g./m.²) although the expenditure of insecticide was identical.

Treatments at 0.3 to 0.5 g./m.² are compared in fig. 4. None of these treatments gave persistence comparable with that obtained in the first two experiments made with dieldrin (by Davidson, and trial 25). The 25 per cent. powder, on actively absorbent pure mud plaster (27), was as good as the 50 per cent. at 25 per cent. higher rate (26). On mixed plasters, of active mud and sand, the

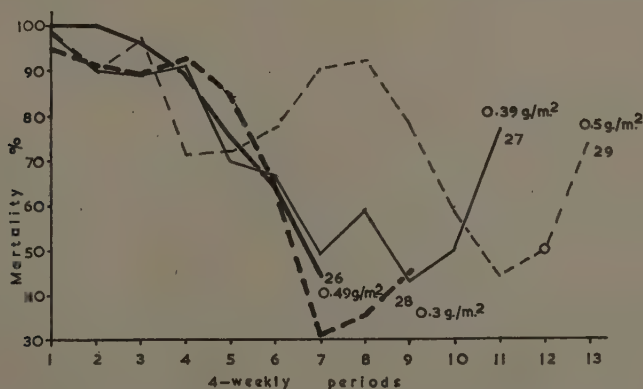


Fig. 4.—Percentage mortality among females of *A. gambiae* and *A. funestus* entering huts with mud walls completely treated internally with dieldrin wettable powders at 0.3 to 0.5 g. dieldrin/m.² (Table IX). Mortalities calculated as in fig. 1. Thick lines show 50 per cent. powders, thin lines 25 per cent. powders. Continuous lines show the mortalities in huts with walls plastered with pure absorbent mud, pecked lines mortalities in huts with a plaster of equal parts of mud and sand. Circles indicate infestation by ants.

50 per cent. powder was applied at too low a rate (28) for comparison to be made. On active mud plaster the 25 per cent. wettable powder (27) was less persistent than on a plaster of mud and sand (29) but the comparison is nullified by low dosage. The 50 per cent. powder on active mud plaster was about as effective as it was on semi-active mud-and-sand plaster at only $\frac{2}{3}$ the rate of deposit (compare 26 and 28). In all these trials, 40 per cent. of the internal surface was non-absorbent roofing material, and any differential effect due to the walls has to outweigh this stabilising feature. There are thus strong indications that 25 per cent. powders are more persistent than those of 50 per cent. on sorptive substrates, and that persistence is greater if the wall is of low activity or only partially composed of active material.

Before the experiments described above were completed, others were started to supplement the information they would provide. Two points in particular needed elucidation. Firstly, it was necessary to find out what is the persistence of dieldrin, at about 0.45 g./m.^2 , on completely non-active surfaces. Secondly, to test it at much higher rates of application in an attempt to duplicate trial 25

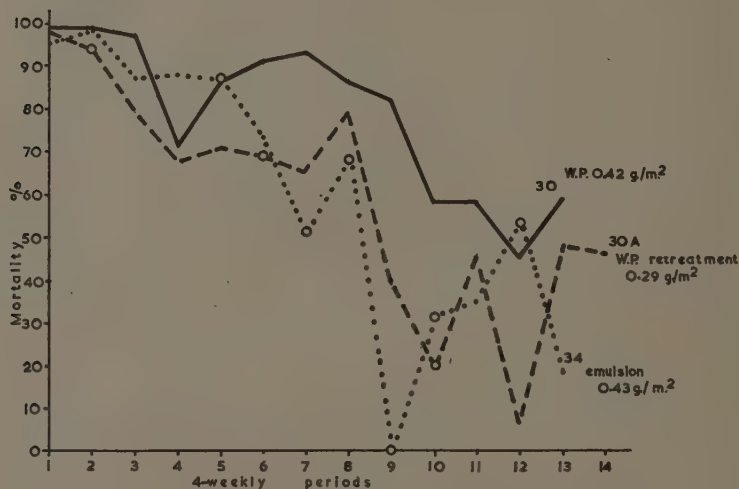


Fig. 5.—Percentage mortality among females of *A. gambiae* and *A. funestus* entering huts entirely with dried banana leaf and completely treated internally with dieldrin. Mortalities calculated as in fig. 1. Circles indicate infestation by ants. The number of each trial, the preparation and the measured deposit are shown (also see Table IX).

and Davidson's trial. The non-active type of surface was represented by banana leaf, which was treated at 0.42 g./m.^2 (trial 30). Comparison of the curves for 29 (semi-active surface) (fig. 4), 30 (inactive surface) (fig. 5) and 26 (active surface) (fig. 4) shows that the first two are similar, apart from temporary fluctuations, but that 26 diverges strongly after five months. This suggests that at $0.42\text{--}0.5 \text{ g./m.}^2$ of dieldrin the mixed plaster is relatively non-absorbent. However, on banana leaf there might be unusually rapid loss as dust from what is a rather flexible surface and for comparison a commercial emulsion concentrate was used; it seemed likely that the wetting agents in the emulsified solution derived from this concentrate would assist in cementing the insecticide to the

surface to a greater extent than those in a powder, while there would be no diluent to mask the insecticide. The deposit of 0.43 g./m.^2 was very close to that of the wettable powder but the emulsified solution was less effective than the powder (trial 34; cf. 30, fig. 5). Ants plagued this trial, but if their effect is eliminated by comparing mortalities in the window-trap catches alone, these are found to be equal for the first six months, after which the powder gives rather higher kills.

The most likely explanation of the discrepancy between the early trials and later attempts to duplicate them is that the deposits obtained in the former were unexpectedly high. This would be possible if, while spraying, the nozzle had been kept at about one foot from the wall, or if the pressure used was lower than usual, assisted by particular care to agitate the suspension. The early dieldrin powders were of such extraordinarily and obviously poor suspensibility that this last factor is probably of importance. Recoveries of 75 per cent. were possible in these circumstances, in which case the mean deposits might have been as high as $0.65\text{--}0.80 \text{ g./m.}^2$. Two huts were treated at an intended 0.80 g./m.^2 , one plastered with mud and sand and the other with plain mud.

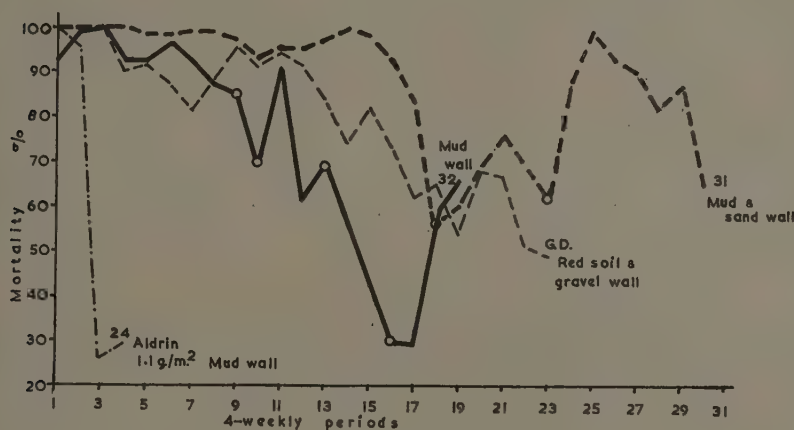


Fig. 6.—Percentage mortality among females of *A. gambiae* and *A. funestus* entering huts with mud walls completely treated internally with dieldrin 25 per cent. wettable powder at $0.8\text{--}0.9 \text{ g./m.}^2$. Also shown are the treatment made by G. Davidson at an unknown rate (Table IX) and a trial with aldrin wettable powder at 1.1 g./m.^2 (Table VIII). Mortalities calculated as in fig. 1. Circles indicate infestation by ants.

The hut with mixed plaster (trial 31) proved extremely lethal for a very long period, exceeding Davidson's experiment. There was a fall in the second long dry season (see later section), assisted by ants, but this was followed by a considerable increase in the following rains, which was maintained (fig. 6) to the forced termination of the trial. The hut with a pure mud wall (trial 32) was much less successful (fig. 6). Part of the fall in kills was due to ants, and after a year the total catch in the hut was very low indeed—in the 14th and 15th months insufficient to calculate a mean kill. The hut became very dilapidated towards the end, and this, as much as low mortalities, led to the early closing down of this trial. The effect of ants can be eliminated to some extent by comparing the mortality of mosquitos caught in the window traps in the two tests. This mortality was consistently higher in the hut with mud-and-sand

plaster. Mean mortalities for successive six-month periods were 98, 82 and 85 per cent. for mud-and-sand plaster, and 83, 47 and 56 per cent. for pure mud plaster. Particulate action was also more persistent in the former hut, the last test for which it was significant being made in the 15th month, when a mortality of 100 per cent. was obtained near the wall and nil in the middle of the hut. In the other hut no significant kill was found after the seventh month, when mortalities near the wall and in the middle were 50 and 25 per cent., respectively (Table X). These tests were made on 2nd July and 4th June 1954, thus seasonal differences are discounted. Contact tests continued to give high kills until the wall panels disintegrated. It is thus possible that the differences were due largely to the relative quantity of air-borne particles produced in each hut.

Therefore, to obtain the exceedingly persistent high kills obtained in early trials with deposits of unknown density, it has been necessary to apply dieldrin at the rate of 0.8–0.9 g./m.². At this rate, persistence in a mud-walled house was only half that in one with semi-absorbent walls. The mud used in the former huts was not of high activity and the lack of persistence of this heavy application seems disappointing. It was an unsatisfactory test owing to extremely small catches in the later stages, the depredation of ants and the dilapidation of the hut. It is unfortunate that no trial was made at a high rate of deposit on highly active material, but some estimate of its effect can be made from discussion (see pp. 663–664) of one of the "retreatment" experiments described later. However, it is clear that, for persistent high kills on any surface beyond the ninth or tenth month, application rates of 0.45–0.55 g./m.² are not sufficient.

The results obtained in all tests for particulate action carried out in dieldrin-treated houses are given in Table X. The duration of this effect is irregular, some of the irregularities being undoubtedly due to seasonal effects (see later section and Table XII). Contact tests were less irregular and gave no evidence of loss of killing power by the walls before the roofs. The wall plates usually disintegrated while still lethal.

(viii) *Repeated applications* (figs. 5 & 7).

If insecticide resting on an external surface is removed to the interior by sorption it is reasonable to suppose that the outer layers of substrate at first become saturated and that further absorption depends on diffusion into the interior from the saturated layer. That absorbed DDT gradually passed into mud, to a depth as great as 1 cm. in 6 months, has been shown by Barlow & Hadaway (1955). In 12 months the insecticide was distributed almost uniformly through the total thickness of 1.2 cm. of their blocks. Presumably, if a second spraying of insecticide is made before the first has all disappeared, adsorption will be slowed down by earlier saturation of the surface and will be further hindered by remains of diluent on the surface with the result that the persistence of the second application will be increased. If the chemical is lost by volatilisation (BHC or aldrin) or mechanically from a non-absorbent surface, there will be no such augmenting effect. Where this effect is present it might be used either to prolong the intervals between treatments when application rate is kept constant, or to economise in insecticide by using a lower application rate. These hypotheses have been tested. Unfortunately ants seriously interfered with the BHC treatment (Table IV, 11A) and it can only be called inconclusive. The dieldrin trials were more fortunate. The banana-leaf hut was used to represent the non-absorbent surface (Table IX, 30, 30A & fig. 5). There was some intermittent trouble from ants but before it developed it was evident that the retreatment, at somewhat less than the original rate, was rather less effective. It may be remarked that at the second treatment some insecticide must have remained from the first, since mortalities were still in the region of 50 per cent. before respraying, and the total insecticide present was nearer to that of the

TABLE X.
Percentage mortality in tests for particulate action carried out in dieldrin-treated huts.

Expt. Ref. No.	Position*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
		Mortality in 4-weekly periods from date of spraying (corrected for mortality in the controls)																
25	Wall Middle	100	100	—	—	100	—	100	—	—	—	33	—	—	—	—	—	—
		100	100	—	—	100	—	100	—	—	—	44	—	—	—	—	—	—
26	Wall Middle	100	100	—	100	—	100	0	10	—	—	—	—	—	—	—	—	—
		100	100	—	76	—	0	62	0	—	—	—	—	—	—	—	—	—
27	Wall Middle	100	—	100	—	14	0	0	0	6	14	0	—	—	—	—	—	—
		100	—	95	—	4	0	0	0	0	14	0	—	—	—	—	—	—
27A	Wall Middle	100	100	32	—	100	—	19	0	—	0	—	0	5	0	—	0	—
		100	100	41	—	9	—	24	0	—	0	—	0	0	0	—	0	—
28	Wall Middle	100	—	37	—	0	5	0	0	—	—	—	—	—	—	—	—	—
		100	—	100	—	13	0	0	0	—	—	—	—	—	—	—	—	—
29	Wall Middle	—	100	0	—	25	0	52	0	5	58	15	—	0	—	—	—	—
		—	100	0	—	0	0	41	0	0	0	16	—	0	—	—	—	—
29A	Wall Middle	—	100	—	—	0	0	0	0	—	100	0	100	—	—	—	—	—
		—	—	8	—	0	0	0	0	—	44	0	0	—	—	—	—	—
30	Wall Middle	—	100	100	100	100	100	15	—	5	0	0	—	—	—	—	—	—
		—	100	100	100	100	100	10	—	15	0	0	—	—	—	—	—	—
30A	Wall Middle	100	100	100	—	70	20	35	—	15	53	25	25	5	—	—	—	—
		100	100	95	—	55	25	5	—	21	15	20	50	11	—	—	—	—
31	Wall Middle	100	100	93	100	100	100	100	100	24	71	19	84	—	68	100	—	0
		100	100	100	100	100	100	100	100	—	63	32	69	—	70	0	—	0
32	Wall Middle	100	—	100	100	—	100	50	0	—	10	5	20	—	0	—	37	30
		100	—	100	100	—	100	25	20	—	15	5	5	—	10	—	10	29
33	Wall Middle	—	100	—	0	—	0	0	0	0	10	—	0	—	28	—	—	—
		—	100	—	0	—	0	0	0	0	25	—	0	—	18	—	—	—
34	Wall Middle	100	100	—	100	—	40	10	—	0	0	20	0	—	—	—	—	—
		100	100	—	100	—	30	0	—	15	5	5	10	—	—	—	—	—

* See text p. 638.

first trial than appears by a mere comparison of the amounts applied at the two sprayings.

The effect of retreatment of an absorbent surface at the same rate of application as the original is shown in trials 27 and 27A (Table IX & fig. 7). Kills of 80 per cent. and over lasted twice as long after the second application. It is

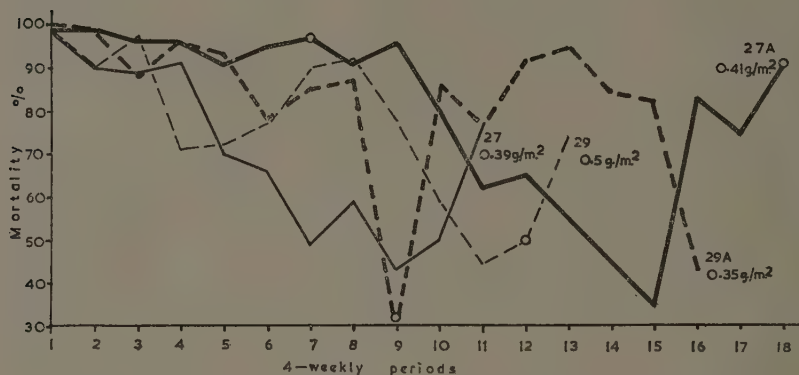


Fig. 7.—The effect of the residue of initial treatments on the persistence of second treatments of dieldrin in mud-walled huts. Huts completely treated with dieldrin 25 per cent. wettable powder. Mortalities following initial treatment shown by thin lines, mortalities after retreatment by thick lines. Continuous line, experiments 27 and 27A (pure mud wall), pecked line, 29 and 29A (wall plastered with equal parts of mud and sand) (Table IX). Mortalities calculated as in fig. 1. Circles indicate infestation by ants.

not possible to say whether the original treatment would have shown an equally high kill after the initial fall; in any case the fall occurred after a much shorter interval. Retreatment of a semi-absorbent surface at 30 per cent. lower than the original dosage is shown in trials 29 and 29A. Apart from the ninth month (dry season fall plus ants) the treatment was remarkably effective (fig. 7). There was no opportunity to make a similar trial in a hut with pure mud plaster, but we would expect that such a plaster would absorb a greater proportion of the deposit than did the mixed plaster of trials 29 and 29A. Accordingly, the augmenting effect should be correspondingly greater. It is not suggested that in this case retreatment at 0.35 g./m.² will last a year, as it did in trial 29A on mixed plaster, but that it will last the six to eight months of the first treatment on active mud at 0.4–0.5 g./m.² (trials 26 and 27), i.e., the second spray can be at 75 per cent. of the strength of the first without loss of persistence. There has been no opportunity to test DDT for the augmenting effect but it would be expected to act in a similar way to dieldrin.

It has been suggested that the superior persistence of the second application is merely an additive effect, the residue of the first application being added to the newly deposited second. If this were all that occurred, one would expect active and non-active surfaces to behave similarly. In particular, trial 30A should be almost as effective as 27A or 29A (the mean deposit rates on test papers being similar, though rather less in 30A than in the other two), for, before respraying, kills in 30 and 27 and 29 were of the same order. In fact, although in the deposit rates in the second application there is some discrepancy between the three huts in regard to the roof, there is less in regard to the walls, and 27A (active walls)

shows very much more, and 29A (semi-active walls) considerably more, persistence than 30A (banana-leaf walls).

(ix) *Partial treatments* (fig. 8).

The laboratory discovery of sorption suggested that most of the prolonged action of a non-volatile insecticide such as DDT or dieldrin was exerted by the deposit on the roof which is of non-absorbent material, particularly since the species concerned show a preference for resting there (Davidson, 1953). Several tests have been made with partial spraying, and there is no doubt that spraying part only of a house with DDT or dieldrin is not efficient (*cf.* McCauley, Fay & Simmons, 1948, who reached the same conclusion when working on *A. quadri-maculatus* Say on a field scale).

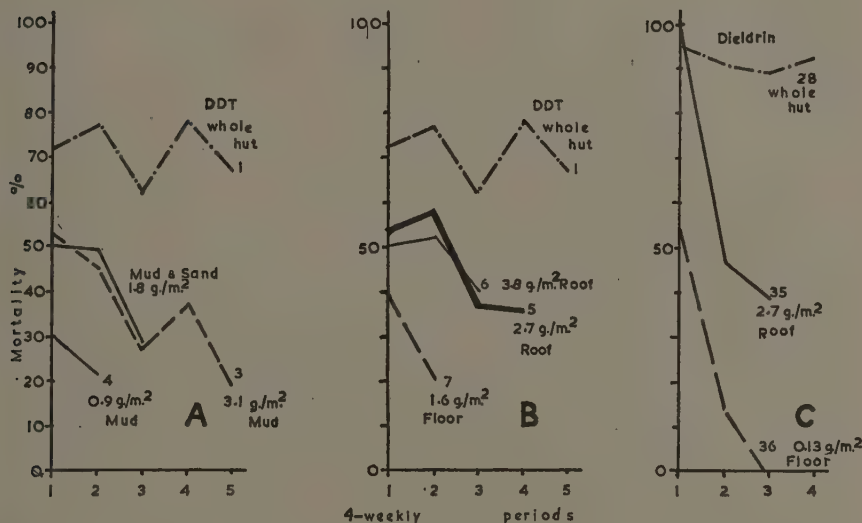


Fig. 8.—Partial treatments, in which only walls, roof or floor of a hut was treated with insecticide. Appropriate complete treatments are given for comparison. A, wall only treated with DDT wettable powder. B, roof or floor only, same treatment. C, roof or floor only, treated with dieldrin wettable powder. See also Tables III & IX. Mortalities calculated as in fig. 1.

In two experiments the roof alone was treated with DDT (Table III, trials 5 & 6). In neither trial was the floor covered. The dose was high in both cases and in both the kill was less than when the whole house was sprayed (*cf.* first two lines of Table III) and deterioration was definite but was slower than when treatment was confined to the wall. Airborne particles were important in the seventh week in the case of 5 but negligible in 6.

Dieldrin was also tried on the roof. It was thought to be so much more deadly than DDT (especially in the production of airborne dust) that it might prove more effective as a partial treatment. The first month's results appeared to bear this out but there was a sudden fall in the fifth week (Table IX, 35 & fig. 8C). When tested in the sixth week the effect of airborne particles was negligible.

Several tests have been made with DDT applied to the wall alone. Laboratory

work had indicated that this would be quite ineffective after a few weeks (Hada-way & Barlow, 1949, 1952). For the first trial 1.8 g./m.² was applied to a mud-and-gravel wall (Table III, 2). Kills were less than when a whole hut was sprayed (G. Davidson, 1953) but did not fall very rapidly. The trial was repeated twice on highly active plaster, the dose in one case being about 3 times that of the other (Table III, 3 & 4). The initial kills differed—the maxima, in the first week, being at the high dose 75 per cent., and at the low dose 42 per cent. With the low dose, mortality fell rapidly to 23 per cent. in the second week whereas there was no considerable drop in effectiveness of the high rate for two months. Thus the higher rate gave higher initial kills and greater persistence. The medium rate on semi-absorbent walls (2) is not strictly comparable since, being an early trial (see p. 633), the floor was not protected during spraying, although the amount of insecticide falling on the floor from a wall application is not large. Comparison with the other wall treatments, making due allowance for dust from the floor (7), shows that the sand in the plaster increased persistence to the extent that 1.8 g./m.² was as effective as 3.1 g./m.² on an active substrate.

BHC was used to treat the absorbent walls alone of a hut after it had been demonstrated that the long-lasting effect of BHC in a hut with mud plaster was due principally to that part of the dose that had been applied to the walls. The trial was wrecked by ants (Table IV, 14). Dieldrin was not applied as a partial treatment to walls.

A number of trials were made to determine what the effect might have been on early experiments of insecticide that fell on the unprotected floor (fig. 8B). This deposit was assessed in several of the ordinary hut-sprays and then an equivalent amount was sprayed on to the concrete floor of a clean hut. With dieldrin, 0.13 g./m.² on a concrete floor gave a 54 per cent. kill in the first month, falling to nil in the third (Table IX, 36). This would have no effect in whole-hut treatments, since mortalities near 100 per cent. are maintained beyond the third month. DDT at 1.6 g./m.² (Table III, 7) was surprisingly effective for three weeks but kills fell to 15 per cent. by the sixth week. Dieldrin on a mud floor was also surprisingly lethal for a short period (Table IX, 37). In all these trials mosquitos exposed in cages suspended at 6 in. and 6 ft. from the floor were killed. It is concluded that DDT and dieldrin on the floor has not affected previous experiments since floor treatments become quite innocuous long before whole-hut treatments show any appreciable falling off in effect; nor will it have any important effect in native huts treated for mosquito control. In the case of BHC, insecticide on the floor may be of more importance. In most native huts the walls and floor will be of the same material, hence the persistence of BHC on the floor will parallel that on the walls, apart from removal by sweeping, etc. Thus this insecticide will not be lost but, acting as a vapour, will be a reservoir of insecticide reinforcing that on walls and roof.

It is thought that the high initial kill when huts are partly treated is due to airborne dust. The importance of this is shown by the floor trials in which the entire kill is probably due to this alone. As the production of airborne particles becomes less the kill falls to a level natural in a shelter only partly treated with an irritant insecticide. In the case of the absorbent-surfaced wall, effectiveness is further reduced by sorption. Unpublished laboratory trials indicated that wall surfaces cease to produce airborne particles in lethal quantities before roof surfaces and while they are still exceedingly lethal by contact. Since at Taveta both species have a preference for settling on the roof (Davidson, 1953) kills of about 30 per cent. are quite as high as could be expected in huts with only the walls treated. It should be noted that *A. gambiae* and *A. funestus* may show a very marked preference for settling in lower parts of huts under certain East African conditions (Smith, 1955) and in such a case partial spraying of the roof alone will be even less effective than it was at Taveta.

TABLE XI.
The effect on the percentage mortality of female mosquitos of introducing clean surfaces into
huts treated with dieldrin wettable powders.

Expt. Ref. No.	1952													
	Mortality in week ending :—													
	May			7	June					12	July 19	Aug. 26		
10	17	24	31		14	21	28	5						
25	{	99	96	95	91	69	85	77	83	72	60	55	54	
		99	96	95	91	94	98	95	95	94	94	79	89	84
		97	50	87	97	47	100	100	70	80	71	57	54	61
Davidson's dieldrin trial	{	100	76	94	97	58	100	100	87	87	80	78	61	

The second and fourth lines give the complete mortality, the first and third lines mortality when morning window-trap and hand-catches are kept only 7 hours (see text, p. 638). All corrected for corresponding mortality in the controls.

(x) *Nature of the fumigant-type action of dieldrin and BHC.*

A test was made using filtered air in a dieldrin-sprayed house, and it was found that one thickness of Watman No. 1 filter paper rendered the air harmless to *A. gambiae* and *A. funestus*. The results have been published elsewhere (Davidson & Burnett, 1952). Laboratory trials showed that the vapour of BHC readily passed the filter paper. It was concluded that the fumigant-type action of dieldrin is due to airborne solid particles. A better term therefore to describe the distant action of non-volatile insecticides is particulate action (Davidson, 1953). This explains the irregularity and unpredictable effect of this action, since the production of such particles presumably depends on substrate and formulation, among other possible variables.

(xi) *The effect of introducing clean surfaces into treated huts* (Table XI).

When it was established that dieldrin produced airborne solid particles it seemed likely that these would settle on and contaminate clean surfaces. This, and the effect on mortality of mosquitos of introducing an extensive clean surface into a sprayed hut, were investigated by the introduction, in June 1952, of clean hessian cloth into two dieldrin-treated huts, that of the trial started by G. Davidson in March 1951 and that of trial no. 25 of the present series. One piece of cloth was hung vertically from the roof ridge (area 32 sq. ft.), and others to form a partial false ceiling of 80 sq. ft. area. Since both sides of the vertical cloth would be utilised by mosquitos for settling, but only the lower surface of the ceiling, the total clean area was about 140 sq. ft., compared with 400 sq. ft. of treated surface (160 roof, 240 walls). The weekly mortalities in the two huts are given in Table XI. The results are complicated by the seasonal fall in mortality experienced at this time of the year. The introduction of the cloth caused an immediate fall in the early kill in trial 25—the final kill showed no decrease. It appears, therefore, that the mean dose picked up was smaller, or more insects picked up only a small, (but ultimately lethal), dose. The effect was the same in Davidson's hut but less lasting. Removal of the cloth brought a further drop in mortalities. This has been interpreted as follows:—the initial introduction of the clean resting surfaces permitted the mosquitos to rest safely, and probably restricted air movements which might transport particles of insecticide. Very soon the surface became contaminated and lethal, and mortality would have increased but for the usual seasonal decrease operating to reduce it. On removal of the cloth, a large lethal area was removed and movement of mosquitos was less restricted, *e.g.*, the light from the window trap was no longer obscured from insects on the roof and they could leave with greater facility. The cloth, after removal from each hut, was tested for contamination and was found to be very lethal. It appears therefore that the introduction of clean articles into huts even several months after treatment will have little permanent effect in reducing kills.

(xii) *The effect of wood smoke and tar* (fig. 9).

Unpublished laboratory work has indicated that, at low rates of application of insecticides, wood tar as a substrate would absorb them and render DDT and dieldrin (but not BHC) innocuous. At the usual rates of application used in practice this effect was not important, but a very thin layer, deposited from a smoky fire after the insecticide had been applied, rapidly obscured heavy deposits of both dieldrin and BHC. Since the tar was necessarily deposited from hot smoke it is possible that the BHC was volatilised and not obscured.

Two huts were built, plastered with pure active mud and exposed to a smoky fire day and night for about four months. The result was a faint yellowish deposit on the roof. One hut was then treated with BHC (50% W.P.) at 0.25 g./m.² (γ BHC) (Table IV, 11) the other with dieldrin (25% W.P.) at

0.86 g./m.² (Table IX, 33). Thereafter, the fires were continued in the day-time only. Extra window traps were fitted at dawn and removed at midday to catch any mosquitos driven out by the smoke. None was, in fact, caught, and so the extra traps were discontinued after a while. Total catches gave no indication that the smoke repelled mosquitos from entering the huts.

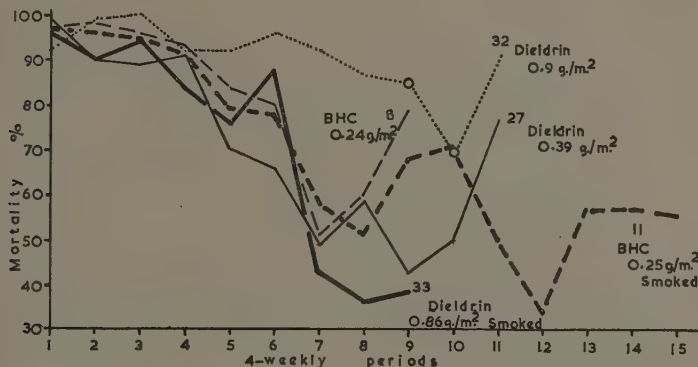


Fig. 9.—The effect of wood smoke on mortality in huts completely treated with BHC or dieldrin wettable powders (Tables IV & IX). Mortalities for treatments subject to smoke shown by thick line, comparative unsmoked treatments by thin line. In the case of dieldrin there is no completely comparable treatment since trial 32, at the same dose as 33, was on a less active substrate. As an additional comparison, 27 has been included—the same substrate as 32 at half the dose of insecticide, giving comparable persistence. Mortalities calculated as in fig. 1. Circles indicate infestation by ants.

The results obtained were not unexpected. The tar had no deleterious effect on the BHC; trial 11 compares well with trial 8 (Table IV & fig. 9). The reduction in the persistence of the dieldrin was considerable. The smoked treatment (33) can be compared with an unsmoked trial at the same dose but on a less active substrate (32) or with another at half the dose and on an identical substrate (27) (Table IX & fig. 9). Ants were not detected at any time in the hut used for trial 33. The particulate effect was very brief, being absent by the fourth month (Table X, penultimate line).

(xiii) Seasonal variation in mortality (Tables XII & XIII).

Fairly early in this series of trials some puzzling instances were noted where the mortality showed a sudden increase after several months of progressive fall (e.g., Table IV, 8; Table IX, 27). In most cases neighbouring huts were in process of reconstruction, or had been recently sprayed, and the rise in kill was put down to a certain amount of generalised contamination by the substance of the demolished hut or accounted for by movement of contaminated mosquitos from a freshly sprayed hut to others nearby. In later trials this rise in kills was seen when there was no possibility of contamination and it was found that it usually coincided with the onset of a rainy season. At the end of the rains, mortality gradually fell, and reached a minimum at the end of the dry season. This was naturally the time at which the treatments were terminated and huts reconstructed since the insecticide was apparently no longer lethal. Catches were maintained to the last; thus in the later houses in a batch they were continued until the seasonal rise in kill had started. There is no question of the variation being due to the increased proportion of *A. gambiae* during the rains,

TABLE XII.

The effect of humidity on the percentage mortality of female mosquitos in cages suspended in the huts, without contact with walls or roof.

Date	1954-55	Oct. 13-14	Nov. 23-24	Dec. 16-17	Feb. 7-8	Feb. 28-Mar. 1	Mar. 28-29
Rain in previous week (in.)		0.33	0	0	3.15	0	1.40
Expt. Ref. No.	Position	Mortality					
23 (BHC)	Wall Middle	64 0	50 32	0 16	100 100	18 12	100 100
11A (BHC)	Wall Middle	0 0	10 24	0 0	100 53	0 0	100 73
29A (dieltrim)	Wall Middle	0 0	0 0	0 0	100 44	0 0	100 —
Control	Wall Middle	0 0	0 0	0 0	0 23	0 0	0 0

(On November 25th-27th, 2.67 in. of rain fell.)

since this species is certainly no more susceptible to insecticides than *A. funestus* (Davidson, 1953, and confirmed in the present series for all three principal insecticides). The phenomenon was not shown by any of the DDT experiments, but some examples from BHC and dieldrin trials which show it have been extracted from the Tables and are quoted below.

BHC (Table IV)

Expt. Ref. No.		
8	—increasing mortality in	March–Apr. 1953 (periods 7–8) fig. 2
9	— “ “ “	Oct. 1953 (period 7)
11	— “ “ “	Oct.–Nov. 1953 (period 9) fig. 9

Dieldrin (Table IX)

27	—increasing mortality in	Nov. 1953 (period 11) figs. 4 & 7
27A	— “ “ “	Feb. 1955 (period 16)
29	— “ “ “	{ Oct.–Nov. 1953 (period 7) figs. 4 & 7 March–Apr. 1954 (periods 12–13) figs. 4 & 7
31	— “ “ “	Feb.–March 1955 (periods 25–26)

It is useful to compare these with the rainfall figures of Table I. In each case the rise in mortality takes place in a month with increased rainfall. This increase, tentatively presumed to be dependent on relative humidity, was well shown by the regular tests for fumigant-type action in February 1955. On February 2nd–8th there was a heavy and unseasonable rainfall of a total of 3.15 in. Tests for fumigant-type action this month were done on the night of February 7th–8th. The results for this date and for the preceding three and subsequent two months are given in Table XII, in which the rise and fall of mortality with the arrival and end of rainy periods is well shown. These results were followed up by making daily tests by suspending a cage of mosquitos in the middle of one of the huts (trial 29A, dieldrin W.P.). The results are given in Table XIII. A hygrograph was installed in the experimental hut but proved unserviceable and there are, therefore, no direct measurements of humidity. However, the correlation between rainfall and high mortality is suggestive. It does not follow that rain at the laboratory and the rain gauge was identical, and discrepancies may be due to this; and it is noticeable that at the height of the rains in April and May kills continue at moderate levels in the absence of rainfall. This is not surprising if the variation is due to atmospheric humidity which is at a generally higher level at this period.

The seasonal effect was not noted in any DDT trial in this series but it perhaps explains the course of one of Davidson's experiments, with DDT oil-bound suspension (Davidson, 1953, Table III). It was certainly found by Hocking (1947) in a tent sprayed with DDT in kerosene solution. Thus the effect has been noted with all three common insecticides on a permeable inactive surface (canvas) and on active mud both pure and mixed with an equal amount of sand. Bordas & Navarro (1955) have recently detected an increase in the particulate action of dieldrin on mud when the atmospheric humidity rose, and Hadaway & Barlow (1952, p. 305) have found that high humidity reduced the rate of sorption of insecticides. Since this paper was completed these latter authors have confirmed that, in the laboratory, an increase of humidity may raise the availability of insecticide and suggested the mechanism by which it operates (Hadaway & Barlow, 1956).

Discussion.

The main aims of this series of trials were to reconcile earlier laboratory and field work and to evaluate dieldrin for the control of adult mosquitos in East Africa; both have to a considerable extent been achieved. A definite relation has

TABLE XIII.

Percentage mortality of female mosquitos in cages suspended in the middle of hut 29A
(treated with dieldrin) during early rainy season 1955.

Date	March 11-19	20	21	22	23	24	25	26	27	28	29	30	31	Apr. 1	2	3	4	5
Rain (in.)	..	—	1.20	—	—	0.6	0.35	0.25	0.20	—	—	—	—	—	—	—	—	—
Mortality	..	0	0	46	75	80	100	100	95	100	60	100	83	19	14	90	94	0
Date	Apr. 6	7	8-11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Rain (in.)	..	—	—	0.6	0.3	—	—	—	—	—	—	—	—	—	0.5	—	—	—
Mortality	..	16	0	No tests	100	100	60	0	0	47	56	40	100	84	100	32	76	42
Date	Apr. 27	28	29	30	May 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Rain (in.)	..	—	—	—	—	—	—	1.15	—	—	0.5	0.45	—	0.14	0.45	—	—	—
Mortality	..	100	95	90	100	80	31	—	57	67	70	—	100	—	45	100	42	20

been shown between substrate and the persistence of insecticide, at least in the case of BHC and dieldrin. This was not clear from Davidson's work for two reasons, the unsuspected lack of sorptive power of the soil he used for lining his huts and the heavy doses of DDT now used in practice for residual control. In fact, the size of the recommended dose is probably due to the fact that it does obscure the sorptive power of the substrate; dieldrin, which is used at one-fifth to one-third of the rate of DDT because of its much higher toxicity, therefore shows the effect much more clearly, but Davidson was able to make only one trial with this insecticide.

Some of the earlier trials with dieldrin at about 0.45 g./m.² and BHC at 0.25 g./m.² showed that absorption was a factor to be reckoned with, and partial treatment of huts with both dieldrin and DDT confirmed that the walls were important in maintaining high kills. Although light deposits were less persistent than heavy ones, absorption was never a matter of days, as in the laboratory. Probable reasons for this were firstly the nature of the laboratory mud which was dried, ground, sieved and generally denatured and reduced to an even consistency. Secondly, the inevitable irregularity of the deposit, with local variations of several times the mean rate. Thirdly the effect of inert diluent in the wettable powders, and fourthly the large crystals of insecticide in all the DDT and dieldrin wettable powders used.

It was hardly possible to line a hut with laboratory-prepared plaster, nor would it be of much practical importance to have done so, but the question of sand in plaster was investigated because an explanation was needed of the exceptional persistence of dieldrin at an assumed rate of 0.45-0.6 g./m.² in the first two trials. It was found that 50 per cent. sand seems to have an important effect in prolonging the action of dieldrin at both high and low rates. At the same time such plaster absorbs BHC and on it dieldrin exhibits the augmenting effect (see p. 654) due to the residue of previous applications which it shows on pure mud, *i.e.*, the mixture has a combination of the characteristics of active and inactive substrates. It was virtually impossible to investigate the effect of irregular deposits because these are inevitable in semi-field experiments. The third question, of dilution, was investigated when trying to account for the success of the early trials and there are strong indications, not amounting to proof, that on active surfaces the 25 per cent. powders were considerably more persistent than those of 50 per cent. The influence of small particle size in increasing absorption of DDT had been investigated by Davidson (1953) and this was not repeated. Unfortunately his walls turn out to have been relatively non-absorbent in any case, but he did obtain evidence of more rapid sorption of small particles. We have to rely on laboratory evidence alone for information on the relation between particle size and sorption by highly active substrates (Hadaway & Barlow, 1952, pp. 285-289). This is sufficient to suggest caution in using the finely divided 50 per cent. wettable powders which satisfy World Health Organisation specifications; considerable sorption has been demonstrated with the exceedingly coarse 25 per cent. formulations used in these trials and a more rapid effect is to be expected with the finer type. Presumably the augmenting effect of the residue will be unaffected since it is exerted on the vapour phase, due to saturation of the surface layers of the substrate. The importance of formulation in producing airborne particles is so far unknown, but since fairly high kills are possible without this effect being detectable its importance in practice may lie in the contamination of clean surfaces introduced into a treated hut. It is notable that one of the first effects of smoke appears to be the suppression of particulate action.

The duration of a given application of dieldrin in practice is discussed in the appropriate section and summarised below. It is unfortunate that we have no direct evidence on the persistence of dieldrin at high rates in huts with walls of

highly active mud because the experiment designed to obtain this information was unsatisfactory for several reasons, but an idea may be obtained from the results of multiple treatments, in particular trial 27 and 27A (Table IX). In this hut a total of 0.8 g./m.² of dieldrin was applied in two equal treatments ten months apart, the second spraying being made on the same day that trial 32 was started with 0.9 g./m.² in a single application. Presumably some of the dieldrin originally applied had been lost mechanically in the months preceding the second treatment and more would have diffused so deep into the wall that it could not affect the rate of absorption of the second treatment. The fall in mortality prior to retreatment confirms that much of the original deposit was no longer at the surface and the effective deposit immediately after the second treatment was no doubt less than the sum of the two sprayings, i.e., less than 0.8 g./m.². Mortalities in the hut were 80 per cent. and over for 40 weeks, and over 60 per cent. for 48 weeks at least. It seems reasonable to expect that a genuine deposit of 0.8 g./m.² applied in one operation would extend the period of kills of 65 per cent. and over to a full year. According to Macdonald & Davidson (1953) this would control malaria in most natural conditions. These authors also state that 85 per cent. mortality should control malaria in the most severe cases. It would be rash to expect, on the present evidence, that this level of efficacy would be maintained by 0.8 g./m.² of dieldrin on a highly active substrate, but it should certainly do so for a full nine months and probably more. In a campaign to control malaria in an area where many houses are lined with highly active mud a first treatment at 0.8 g./m.² should be followed by respraying within a year, but even in the most stringent conditions retreatment need not be considered in less than nine months.

The question of BHC is more straightforward since its action is largely that of a fumigant. On a non-absorbent surface it rapidly volatilises, but, provided the plaster contains active material, the insecticide is rapidly removed from the external surface, and thereafter escapes slowly as a vapour, exerting an unexpectedly prolonged effect. This had already been noted by Downs & Bordas (1951) in Mexico. Contact tests have shown that the prolonged lethal effect of the deposit resides in the walls, although exposures of greater length than usual often demonstrated some insecticide on non-absorbent surfaces even a year after treatment.* The effect of smoke on BHC is not detrimental and might even be beneficial in reducing the rate of volatilisation. The use of a blooming resin for the same purpose does not appear to be worthwhile on mud surfaces, and from the performance of such a preparation containing BHC it is deduced that, with a non-volatile insecticide, results would be less favourable than with wettable powders, certainly if relative costs are considered. The practical importance of the retention of BHC by mud will vary with the circumstances, since a high degree of ventilation is likely to reduce persistence.

The seasonal effect, recently confirmed to be due to changes in humidity (Hadaway & Barlow, 1956), is important. It has been noted in Mexico by Bordas & Navarro (1955). In humid areas an insecticide may show a more uniform and lasting effect than it does in an area with a pronounced dry season. In some localities with a dry season, careful observation over a complete annual cycle may show that one treatment of insecticide revives sufficiently to last two breeding seasons, since these frequently coincide with increased humidity. On the other hand, if breeding takes place in irrigation water, river pools or artificial containers in a dry area and is dependent principally on temperature, a likely state of affairs in East Africa, the efficiency of the insecticide may be at its lowest coincident with the highest rate of mosquito production.

* These tests were done in the laboratory. There is no question of mortality being due to vapour in the air of the hut, as might be the case if tests were carried out in the experimental hut itself.

Conclusions.

It is appropriate to state what recommendations should be made as a result of these trials, but it must be emphasised that they should be interpreted in the light of local conditions. Rebuilding, dilapidation, etc., will all affect the lethality required to give control of mosquitos.

Firstly, there is nothing to alter the conclusion of Macdonald & Davidson (1953) that DDT can control malaria in many natural conditions. Against the common African vectors, the first treatment should be at 2.2 g./m.² and repeated after six months, probably at the same dosage. This insecticide has the advantage of known safety, familiarity, cheapness and availability, and the fact that decrease in effectiveness is slow. The wettable powders usually available in the sterling area are of poor suspensibility.

BHC appears most suitable for short transmission periods of high intensity, since it will last three months, giving high mortalities. In houses with absorbent walls it can persist for ten months, but the effect of increased ventilation is unknown and no augmenting effect is to be expected from successive applications. It is not adversely affected by wood smoke. It is safe, cheap, familiar, usually available locally and rapidly affects a number of domestic pests including ticks and rats. Where the period for which protection is required is short, and therefore the prolonged residual action of dieldrin is not required, it may be as effective but cheaper to use BHC at 1.6-2.2 g./m.² instead of dieldrin at 0.5-0.6 g./m.². For emergency use in epidemics it may be the best insecticide to use, being easily obtained and requiring less supervision in application than dieldrin, which requires safety precautions. It is readily formulated to give good dispersibility. To ensure continuous protection it needs applying quarterly, but if walls are of active mud, spraying may lag behind schedule by up to two months without serious risk.

Dieldrin is the insecticide to use when high kills are required for many months or it is particularly important not to risk any falling off in kill between sprayings, e.g., if elimination is aimed at. Its virtue lies in the unique combination of high kill and long persistence, a fact not always appreciated if a comparison is made on the basis of price alone (e.g., Annecke, 1954). Its disadvantages are relatively high price and the advisability of taking safety precautions during application. Suspensions are known to have a serious acute poisoning effect on fowls, cats and goats. It is the obvious insecticide for large-scale extermination campaigns or routine spraying by skilled gangs in areas with continuous transmission at rates too high for DDT to cope with. In African areas it is probably safe to assume that the majority of walls are at least semi-absorbent and that a spraying cycle of more than a year is inadvisable owing to new construction, cleaning of houses, accumulation of soot, etc. A recommended spraying schedule using wettable powder would be at 0.8 g./m.² for the first treatment, followed annually by 0.55 g./m.², in each case allowance being made for wastage (e.g., with efficient nozzles, expenditure of 1.1 g./m.² and 0.7 g./m.² might be suitable). With a six-monthly spraying schedule, dosages could be reduced to 0.55 g./m.² for the first and 0.35 g./m.² for subsequent treatments (expenditure of 0.7 g./m.² and 0.45 g./m.², respectively). Until there is more information on the persistence of the micronised wettable powders now in use, these dosages should not be reduced.

Spraying should be scheduled so that the last month before renewal falls in a season of low transmission in case the effect falls off rather more rapidly than expected. This is unlikely in many areas of continuous transmission where atmospheric humidity is high. Where a small loss of effectiveness is likely to be serious, the above high rates could be used at nine-monthly intervals with complete safety; attempts to extend this period to a year by increasing the dose

residual applications
 Second applications of dieldrin in huts with mud or mud-and-sand walls were much more persistent than the first treatment. This is here termed the augmenting effect. Economy can be made by reducing either the application rate or the frequency of treatments. On impervious materials there is no such effect. The experiment with BHC was spoiled by ants. DDT would be expected to behave like dieldrin, but no trial was made.

Treatment of part only of a hut (walls or roof) was less efficient than treatment of the whole hut. Insecticide which falls on a concrete or even a mud floor has a strong but short-lived effect, exerted as airborne dust.

It was shown that the fumigant-type effect of dieldrin is exerted by particles too large to pass filter paper, but BHC has true vapour action. Particles of dieldrin will contaminate clean surfaces introduced into a heavily treated hut even 15 months after spraying, rendering them lethal.

Smoke from wood fires quite rapidly renders dieldrin ineffective, particularly reducing the particulate effect. BHC is not adversely affected.

A pronounced effect of season on mortalities was found, which appears to be related to atmospheric humidity, the effect of rain falling in otherwise dry periods being particularly marked. This may change the results of tests for fumigant or particulate action from zero to complete kills.

It is concluded that DDT, BHC and dieldrin all have their uses in control of Anophelines and in public health work generally, and suggested treatment schedules for them are given. It is noted that the highly-divided dieldrin formulations prepared according to World Health Organisation specifications may be found less persistent on active surfaces than the crude preparations available for these trials.

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OBSERVATIONS ON BIOLOGICAL VARIATION IN *ORNITHODOROS MOUBATA* (MURR.) (ARGASIDAE) IN EAST AFRICA.

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Investigations into the "distribution and bionomics of *Ornithodoros* ticks in relation to relapsing fever" commenced in June 1948 under a Colonial Development and Welfare Research Scheme. All observations have, however, been confined to *Ornithodoros moubata* (Murr.) as this is the only common species with which the human population is likely to have frequent contact both in dwellings and in the field. During the first tour of three years, all observations were restricted to the smaller and isolated endemic foci of relapsing fever in Kenya Colony, as these provided an opportunity for study of a series of closed environments of a more concise nature than could have been found in Tanganyika.

When these investigations started, there had been no suggestion that *O. moubata* could be anything but a single biological entity. This paper is a record of observations that have led to the conclusion that there may be three biological forms of the species in East Africa. The greater part of the paper is concerned, however, with the probable existence of two forms in African huts.

This tick had earned for itself an almost mythical reputation of indestructibility. It had a remarkable resistance to most insecticides, heat, desiccation and starvation. It occurred from sea level to 9,000 ft. (Heisch, 1950) and had a wide distribution in Africa (fig. 4).

The literature, and popular opinion, has a strong "urban" flavour, no doubt engendered by the concern of health authorities with the presence of the tick in camp sites, prisons, schools, rest-houses and native hotels in which the ticks acquired an unnaturally high level of infection and from which it is often impossible to remove them. The present investigation has shown that the true centre of infestation lies in the hut homes of the African peasants in the large native tribal areas where tick-infested dwellings can be numbered in tens of thousands. Throughout the present studies, only these rural areas have been investigated since it was considered that urban infestation would provide an artificial environment and give misleading information. It should be stated here

that, since the first concern of the investigations was the distribution of the tick and its relation to relapsing fever, the biological observations described are of a generalised nature and a by-product from the application of a routine method of hut examination made in a total of 4,638 huts taken as a completely random sample during the surveys of different parts of East Africa. The investigations have only recently reached a stage where selective methods of investigation have become possible.

Another impression conveyed by the literature is that *O. moubata* favoured hot and rather arid conditions. Although these investigations have not included the lightly populated semi-arid country of central Tanganyika, it has been unusual, within the area studied, to find tick infestations in huts in which the relative humidity of the tick habitat during the dry season was below 60 per cent. Most infestations have been found at relative humidity (R.H.) values of over 70 per cent.

Before proceeding, it should be mentioned that African tribal characteristics, and in particular their overall housekeeping practices, take precedence over all other factors affecting tick infestation.

Bantu tribes have low levels of household hygiene and are frequently tick-infested, while Nilotic, and Nilo-Hamitic tribes are largely free.

These factors and the subject of the epidemiology will be dealt with in further publications. The results of individual surveys are appearing in the *East African Medical Journal*.

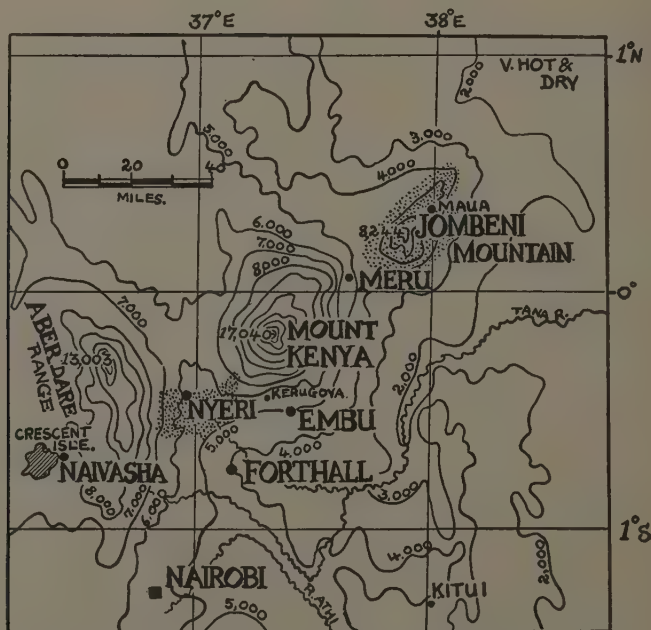


Fig. 1.—Sketch map of the Mount Kenya area of central Kenya showing the position of the endemic foci of relapsing fever in Nyeri and Meru Districts (stippled), Embu District and the Naivasha site of animal burrows from which *O. moubata* used in experiments (see p. 705) were collected.

The overall picture of the problem of biological variation in the regions studied will now be briefly summarised under the headings of Kenya and Tanganyika.

Relapsing Fever and *O. moubata* in Kenya Colony.

In Kenya Colony, relapsing fever was endemic in three areas of heavy rainfall, associated with high mountains, and this suggested that permanent tick infestation might be dependent upon the presence of an assured high humidity. Consequently, the first investigation was made in Meru District of the Mount Kenya area (fig. 1) to appraise the general situation and work out appropriate survey methods.

Meru District.

This large tribal area with a population of just over 300,000, is situated on the NE. corner of Mount Kenya and includes a separate mountain rising to an altitude of 8,244 ft., called the Nyambeni or Jombeni range, where the rainfall is exceptionally heavy and reaches a maximum of 120 in. per annum. The visit was deliberately arranged to coincide with the height of the rains to observe the reactions of the tick to humid conditions, should it be found to occur in association with them. It was found that 27 per cent. of all African huts in the Jombeni mountains, and certainly to an altitude of 7,000 ft., were infested with *O. moubata*, and it was estimated that 10,000 huts were infested with these ticks in the area (Walton, 1950).

The mean relative humidity of the microhabitat in 21 infested huts, measured at the point of greatest tick concentration, was 85 per cent. and the temperature 72°F.*

Nyeri District.

The methods developed in Meru were next applied in an extended and detailed survey of the Nyeri Kikuyu Reserve which lies between Mount Kenya and the Aberdare Mountains at an altitude of 5,000 to 7,000 ft., and normally receives a rainfall of 50 in. per annum. Unfortunately the rains (which should have amounted to at least 10 in. during the time it took to survey the area) failed completely for the first time on record. *O. moubata* was present in 12 per cent. of an estimated total of 63,000 huts. Concentrations reached 35 per cent. in some of the wettest and highest locations. The mean R.H. of the tick microhabitat, that is, in the soil, dust-filled cracks and holes in the floors, of 77 infested huts, was 79 per cent. and the mean temperature 71°F.

Embu District.

Before arriving in Meru, a survey had been made in Embu District, lying to the south of Meru and adjoining Nyeri (see fig. 1). This is an area of low endemicity in which tick infestation is very rare. Much of the low-lying country is dry and hot but the higher country receives a heavy rainfall. Measurements of microclimates of apparently suitable but tick-free huts in the low country gave temperature readings as high at 80°F. and R.H. as low as 55 per cent. The presence of a few ticks found in this low country was, without doubt, due to their accidental introduction from Meru and Nyeri. Most infestations appear to last two or three years and then die out, unless reinfestation occurs, as is the case in a few native hotels lying on the main routes of travel. These probably act as focal points for spread of infestation.

Taita District.

A third survey was made in the Taita Hills, a precipitous mountain rising out of the hot dry plains to an altitude of approximately 7,300 feet, 100 miles inland

* The Fahrenheit scale was used for convenience and speed of work within the short range of variation found in African huts. Readings were made to the nearest whole figure. Conversion is given in fig. 7.

from the Kenya coast (fig. 2). Relapsing fever had been known to occur in the area for over 25 years. In the hot foothills, where the R.H. of the floors of the huts was as low as 62 per cent. and the temperature as high as 80°F., ticks were almost entirely absent despite their abundance at the higher altitudes. As shown

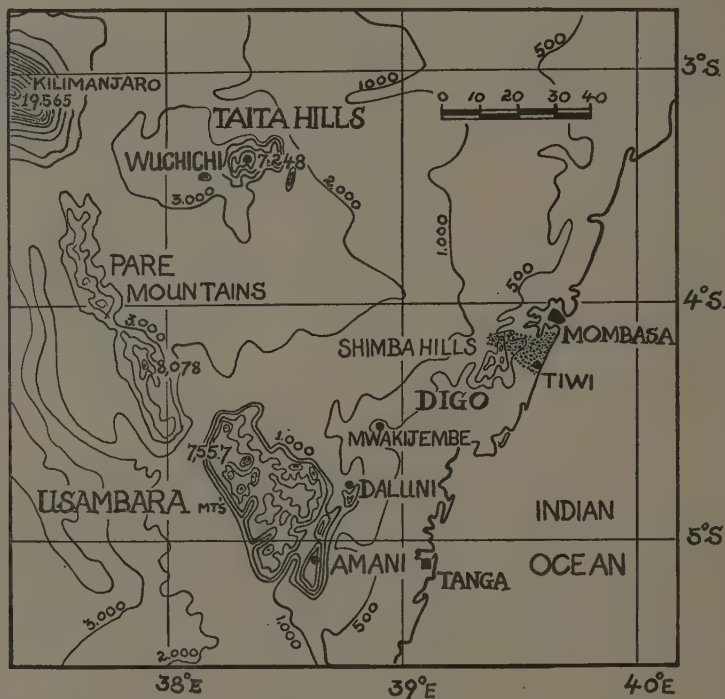


Fig. 2.—Sketch map of the SE. corner of Kenya and NE. corner of Tanganyika showing the relative positions of the Taita Hills, Usambara Mountain area and Digo District (tick-infested area in Digo stippled).

in Table I, the infestation increased towards the summit where the rainfall exceeds 60 in. per annum. The mountain was estimated to contain over 4,000 infested huts. The mean R.H. of the floors of 28 infested huts was 85 per cent. and the temperature 71°F.

It could be inferred from the close agreement between the microclimatic data in Meru and Taita, coupled with the inability of the ticks to establish themselves in the hotter and drier neighbouring country, that these Kenya highland ticks require a high humidity and are apparently unable to colonise huts successfully in hot and dry conditions.

Digo District.

Before starting these investigations it was known that *O. moubata* was abundant in the hot and humid Digo District to the south of Mombasa on the Kenya coast. Whereas in the cool highlands the incidence of relapsing fever and

the abundance of ticks was roughly in agreement in all areas, at Digo the incidence was greatly reduced. The incidence of recorded cases of relapsing fever per 1,000 per annum was only 0.06 in Digo; in Nyeri it was 1.0, in Taita 0.7 and in Meru 1.2.

Digo District was visited in 1950. Ticks were present in a well-circumscribed area of some 80 square miles, in which the maximum infestation rate of houses

TABLE I.

Abundance of *O. moubata* in native huts in altitude zones of 600 ft. in the Taita Hills of Kenya.

Altitude zones	Number of huts sampled	Huts tick-infested (%)	Mean no. ticks per hut	Mean R.H. (%) of tick microclimate
Above 4700	105	41	7.6	88
4100—4700	135	38	4.2	87
3500—4100	70	31	2.5	81
Below 3500	85	3	0.5	68

near the coast was 90 per cent., and not rising, but falling to 55 per cent. in the Shimba Hills further inland. Ticks were absent both in the sandstone country of the hinterland and in a large part of the coastal land where the rainfall was high and the relative humidity of the usual tick microhabitat was over 90 per cent. (Walton, 1955).

Excluding the sandstone as otherwise unsuitable to tick infestation, it was clear that the ticks were only present in that part of the area with the lowest humidity—where the mean R.H. of the microhabitats in tick-infested huts was 77 per cent. and the temperature 80°F. In the few huts that were not infested the mean R.H. was 89 per cent. and the mean temperature 77°F.

These ticks, compared with those found in the highlands, show a reversal in their choice of microclimate.

A study of the few cases of relapsing fever recorded in Digo District made it clear that the human population was very rarely infected by the ticks despite their great abundance. The people treated the presence of the ticks in their houses with complete indifference and stated that they were rarely bitten by them. Either the ticks were uninfected or they fed on some source of blood other than man. It was considered that domestic fowls might be the most likely source of food, since they were quite common in the houses, and goats and sheep were not. In some houses, the ticks appeared to be associated with the fowls, but this association was not an obvious one; they were never found on a fowl and rarely found in a fowl's nest although they had previously been found in similar situations in the Taita Hills and in Nyeri on numerous occasions.

Fowls were only slightly more numerous in Digo than they were in the Taita Hills, and moreover the ticks were frequently found nearer to the fowls in the small Taita huts than they were in the larger Digo houses.

The behaviour of these ticks when compared with those found in the highlands certainly justified further studies. That these differences could be a product of the high temperature at the coast was certainly unlikely in view of the fact that ticks did not occur at similar temperatures in the foothills of the Taita Hills, and were generally absent over all such hot country throughout the remainder of Kenya. Could these ticks be different from those living in the highlands?

Precursory examination of them did not encourage such an idea as no obvious morphological differences could be detected. Moreover, the R.H. at which they lived was only 8 per cent. lower than the corresponding mean value found in the highlands. There was, however, a big gap between the uniformly lower temperature prevailing in the highland areas of infestation and the higher temperatures with which the coastal ticks were associated. It was considered that further studies in tick-infested country with intermediate temperatures might be helpful, and investigations were needed in other areas where ticks occurred in the absence of relapsing fever. It would be necessary, in future, to keep strictly segregated stock colonies of ticks from the different areas with a view to future detailed study.

Many Africans interviewed in Digo District were emphatic that *O. moubata* was abundant in the foothills of the Usambara Mountains, 80 miles to the south in Tanganyika. Records of relapsing fever from that area were almost non-existent. In 1952, there were only 8 cases recorded from the whole of Tanga Province, and only six of these were traceable to the Usambara area. It was justifiably assumed that the relapsing fever picture in the Usambara area would closely resemble that found in Digo District, and it was decided to visit the area. The Usambara Mountains lie in the same latitude as Tabora in Tanganyika, and are well south of Mount Kenya.

Relapsing Fever and *O. moubata* in Tanganyika Territory.

In several respects the picture of relapsing fever in Tanganyika is very different from that seen in Kenya. Over the whole of the two territories the incidence per 1,000 per annum is roughly 0.54 in Tanganyika, and 0.08 in Kenya. The reason for this difference is obvious, for, whereas the disease is confined to a few areas of high rainfall in Kenya, it is widespread in Tanganyika. The most striking difference is seen in the great abundance of ticks in huts in the dry central areas of Tanganyika and their almost complete absence in similar areas in Kenya. But, in the areas in which ticks occur in Kenya (excepting Digo District), the incidence of relapsing fever is higher than it is in Tanganyika. In Tanganyika, relapsing fever is most abundant in the north-west and the incidence declines towards the south-east, but *O. moubata* is most numerous in the central areas, and this is an important point since the disease has a different distribution from that of its vector. Use is made in Table II of some available data to show that

TABLE II.

The recorded incidence of relapsing fever per 1,000 in certain East African endemic areas compared with the incidence in the Kenya highlands and with the expected incidence.

Area	Observed mean no. ticks per hut	Recorded incidence of relapsing fever	Expected incidence of relapsing fever	Ratio ; expected to observed
A Kenya highlands	3	0.82	0.82	1 : 1
B Lake Province, Tanganyika	11	0.85	3.00	3.5 : 1
C Remainder of Tanganyika	9	0.31	2.50	7.8 : 1
D Central & Eastern Provinces, Tanganyika ..	13	0.25	3.56	14.2 : 1

Data for lines C & D from Phipps (1950) are probably on the low side (p. 684).

a large deficit of relapsing fever exists over much of Tanganyika, compared with what would be expected if the level of endemicity in the Kenya highlands is regarded as a standard for comparison. For reasons discussed further on, the figures of the abundance of ticks in areas C and D in Table II may be regarded as about half the true figures and the ratio between the recorded and expected incidence of relapsing fever in the Central and Eastern Provinces of Tanganyika is then seen to be in the order of about 28:1 and this can be regarded as a most conservative estimate.

Reference to Table III will show that the incidence of the disease varies widely from area to area. In some places like Kilosa it is almost entirely absent, despite the presence of large numbers of ticks. It would seem that the great

TABLE III.

Examples of variation in the recorded incidence of relapsing fever compared with the abundance of *O. moubata* in selected areas of East Africa.

Area	Huts tick-infested (%)	Mean no. ticks per hut	Incidence of relapsing fever, 1953
Mwanza	75	15	633
*Dodoma	69	17	388
*Kilosa	56	11	4
*Itigi	76	19	26
Digo	70	15	1
Usambara	44	15	3
Musoma	6	0.6	48
*Kigoma	36	2	78

* Data on tick incidence of starred areas from Phipps (1950). All data on tick incidence refer to the rural areas outside the townships except Usambara which refers to the Usambara Mountain area.

deficiency of the disease might be accounted for if it occurred in a mild form, or if, for some obscure reason, the tick is not able to transmit the disease as efficiently as it does in the Kenya highlands.

In the Usambara Mountains, a picture was anticipated similar to that seen in Digo District in Kenya, and suspected in similar hot, low-lying places like Kilosa, but it will now be shown that such an assumption was unjustified, for Usambara differed both from the Kenya highlands and Digo District.

The Usambara Mountain area.

The Usambara area consists mainly of a high undulating tableland, surrounded by a precipitous escarpment, and reaches an altitude of just over 7,000 ft. The escarpment varies in height from 3,000 to 4,000 ft. There are two smaller isolated mountains, Amani to the south-east, and Daluni to the east. The whole mountain area is surrounded by hot, low-lying plains and a footpath leads from Daluni to Digo District in Kenya and passes through a watering place called Mwakijembe just short of the border.

The area was surveyed in September and October 1952, making a start at Mwakijembe and Daluni. *O. moubata* was found in 63 per cent. of the huts in this low-lying hot area, and it was also present in huts on Daluni Hill. Domestic fowls were present in 70 per cent. of the huts, and ticks were very numerous. The mean temperature of the tick microclimate in 15 huts was 77°F. and the mean R.H. 79 per cent. *O. moubata* was also found well out in the bush in the burrows of wart-hogs and porcupines.

No ticks were found in huts on Amani Mountain where the rainfall averages around 80 in. The floors of the huts were very damp, the mean R.H. being 93 per cent. and the temperature 71°F.

On the main mountain bloc, rainfall varies widely from 30 to over 80 in., although most areas receive 40 to 50 in. *O. moubata* was widespread and present in 44 per cent. of the sampled huts. On this basis it was estimated to be present in a total of 25,000 huts. The ticks were most numerous above an altitude of 4,000 ft. As the authorities were unaware of their existence and the presence of relapsing fever had been overlooked, it must be assumed that disease occurred in a very mild form.

Many Africans openly boasted of their resistance to infection and they joked about the severity of the fever acquired by strangers to the area. Twenty two cases were traced in one Mission Hospital but these had not been reported. Spirochaetes mixed with malaria parasites were seen in a blood film diagnosed as malaria. The patient, a child, had slept in her grandmother's hut, and 80 ticks were found there. Infections were obtained from five villages by inoculating rats with crushed ticks. Of the 22 cases of fever found in the Mission Hospital records, 5 (23%) were infants, 10 (45%) were between 1 and 16 years of age and 7 (32%) were adults. These figures could imply a level of endemicity similar to that found in Meru and Taita in Kenya (Table IV).

TABLE IV.

Percentage incidence of relapsing fever in age-groups to show the preponderance among children and infants.

District	Infants	1-16	Adults
Taita cases ..	56	136	33
%	25	60	15
Meru cases	88	124	78
%	30	43	27

Several interesting points emerged from this survey. Firstly, it was shown that very heavy infestations can occur and yet remain undetected for entirely different reasons from those met with in Digo District. Secondly, ticks were abundant in the hot foothills and plains, although they were absent in similar situations in the Taita Hills 80 miles to the north in Kenya. Thirdly, ticks were far more numerous than they were in the infested areas in the Kenya highlands. This numerical superiority was entirely confined to the huts containing domestic fowls. When fowls were absent, ticks were no more numerous than they were in the Kenya highlands (Table V).*

* Detailed basic data on the relationship between *O. moubata* and domestic fowls in E. Africa will be incorporated in a further paper that is to be published in due course in the *E. Afr. med. J.*

The association of ticks with fowls had now been seen in hot and wet (Usambara), as well as cool and wet, conditions (Taita Hills). As this association had appeared when the investigations moved southwards, it was tempting to carry out further investigations in the hot and dry country in central and southern Tanganyika. But a certain amount of valuable information was available from

TABLE V.

The effect of the presence of fowls on the abundance of *O. moubata* in huts in the Usambara Mountains.

	Total huts	No. huts tick-infested	Total ticks	Huts tick-infested (%)	Mean no. ticks per infested hut	Mean no. ticks per total huts
Fowls present ..	160	73	2850	46	39	18
Fowls absent ..	70	29	529	41	18	8

these areas as a result of a survey carried out by Phipps (1950), and as half of all the relapsing fever recorded in Tanganyika came from the Lake Province (see fig. 3) alone, it was decided that a survey of that area would link the problem in Uganda, Kenya and the Congo with that of Tanganyika.

The Lake Province.

Topography.—This great area of some 39,000 sq. miles lies round the southern half of Lake Victoria (altitude 3,710 ft. above sea level) and was surveyed by our unit between 16th June and 19th November 1953. To the east and west of the Lake the land rises to an altitude of 6,000 ft., gradually to the east, but more abruptly to the west, and to the south it remains more or less at 4,000 ft. to the boundary, and onwards for the next 300 miles (fig. 3).

The western half, from just west of Shinyanga and the tail of Smith Sound, is clothed with an almost unbroken growth of light forest. In the whole of this area, excepting the northern half of the Bukoba coast, the people are concentrated in pockets of cleared forest, varying in area from 30 square miles to approximately 400 at Kahama. The total population is 2,050,000, and its density, at over 42 per square mile, is double that of any other province in Tanganyika.

Most of the country, however, is thinly populated, with the exception of the Sukumaland* area, and, from Mwanza in a south-easterly direction for 130 miles, and for 60 miles on either side of that line, in almost treeless, rolling, windswept country, live 890,000 people of the Wasukuma tribe, who merge imperceptibly in the south-east with the Wanyamwezi.

The climate and microclimate.—The rainfall varies greatly in different parts of the Lake Province. Broadly speaking, the western half receives over 40 in. per annum, rising to 90 in. along the Bukoba lake-shore. Ukerewe Island, Ukara Island and the interior of N. Mara District receive over 50 in. The western extremity of Ukerewe probably receives over 60 in. In all these areas, only two or three months of the year are dry. All other areas receive a rainfall of under 40 in., and generally under 35. Most of Sukumaland receives 30 in., gradually decreasing both in quantity and reliability in a southerly direction. The dry season progressively lengthens from four months at Mwanza to five and six

* Sukumaland comprises the districts of Mwanza, Kwimba, Maswa, Shinyanga and a large part of Geita which has recently received large numbers of Wasukuma immigrants.

months in Shinyanga District. Further south towards Dodoma, eight consecutive months receive less than 2 in. Whereas the climate is very equable in the wet western region (Bukoba has not recorded less than 64 in.), it is characteristically variable in the drier regions. At Ukiriguru, near Mwanza, the annual rainfall has varied between extremes of 17 and 40 in., and the greatest recorded variations in temperature cover a range of 35°F. In its driest year there were 11 consecutive months with less than 2 in. of rain.



Fig. 3.—Sketch map of the Lake Province, Tanganyika, and adjacent areas.

Turning to the microclimate of the tick habitat inside African huts, and speaking broadly, the day-time air temperature in the whole of the country west of Lake Victoria and including Ukerewe District was 77°F. (mean of 93 observations). In the whole of Sukumaland, Geita and Kahama Districts it was 81°F. (mean of 92 observations).

Temperature inside the tick habitat, that is to say inside cracks in the walls and floors and in loose earth in holes in the floors, follows a slightly different broad pattern. The tick habitat in Ukerewe is no cooler than it is in Sukumaland and Kahama and Geita, and the mean temperature was 75°F. In the whole area west of Lake Victoria, however, it was 71°F.

The humidity of the tick habitat has quite a different pattern. In the whole of Sukumaland, north Kahama and Geita (except its NW. lake shore), the mean relative humidity was 68 per cent. In the mainland portion of Ukerewe District it was 75 per cent. On Ukerewe Island it was 83 per cent., and in the whole area west of the Lake it was 87 per cent. Southwards from Mwanza town there is a gradual downward trend in the humidity of the microclimate of the tick

habitat; in Mwanza District it was 74 per cent., in Kwimba District it was 67 per cent. and in Shinyanga District it was 62 per cent. This trend would continue onwards into the central Tanganyika plateau.

The Lake Province is, therefore, a region in which, to the west, microclimates of the floors and walls of huts are quite similar to those found in the Kenya highland endemic foci of relapsing fever and these merge into an area in which a higher temperature prevails throughout, and in which the humidity gradually falls from 75 to 60 per cent.

In the huts in the wet, western region, however, the mean air temperature was 78°F. (mean of 73 observations), whereas in the Kenya highlands which have exactly the same tick-habitat microclimates, the air temperature was 74°F. (mean of 313 observations). This difference might affect the activity of the ticks on leaving the seclusion of the microclimate of their hiding places and exposing themselves when in search of food. In Maswa District, for some obscure reason, the floors of huts were cool, the mean of eleven observations was 70°F. and one of these was as low as 63°F.

The distribution of the tick.—*O. moubata* is very widespread and abundant in practically the whole of the Lake Province. The overall hut infestation rate based on a sample made in 1,210 huts was 48 per cent., with an overall abundance of 11 ticks per hut (24 per infested hut). A careful note was made of the number of people occupying each hut in a total of 682. This gave a mean of 3.5 persons per hut. Based on the latest available data on native populations, adjusted by the East African Statistical Department, an estimate was made of the total number of huts infested with *O. moubata* in the Lake Province. The number is approximately 280,000.

In all rural areas of Musoma and N. Mara Districts, ticks were virtually absent. A small number of infested huts do occur inland, opposite Ukerewe, in Majita and along the lake shore, and almost certainly also occur northwards along the Kenya shore of the lake. One village on Ukara Island was infested (Smith, 1955), and all the small inhabited islands in the lake are also said to be infested.

On Ukerewe Island, the hut infestation rate was 50 per cent; it was 60 per cent. on the mainland portion of the District. In the whole of Sukumaland and that part of Kahama and Geita Districts occupied by the Wasukuma or Wanyamwezi tribes, the huts were uniformly heavily infested at 72 per cent. The only exception was Maswa, and here 29 per cent. of the huts were infested.

Thereafter, a progressive reduction in the infestation rate occurred in a westerly direction as far as the Ruanda-Urundi border as shown in Table VI.

In Bukoba District, the infestation rate was 59 per cent. in the wet, coastal areas and fell to 44 per cent. in the highlands of Karagwe towards the boundaries

TABLE VI.

Progressive decline in percentage tick-infestation rate of huts across 250 miles of the Lake Province in relation to altitude and rainfall.

	Sukumaland, E. Kahama & E. Geita	W. Geita & W. Kahama	Biharamulo	Ngara
Rainfall (in.)	30-40	35-45	35-50	35-55
Mean altitude (ft.) ..	4000	4000	4500	5100
Infestation (%)	72	64	54	40

Rainfall and altitude data are approximations.

of Uganda and the Ruanda-Urundi to the west, and to 42 per cent. along the Uganda border in the north.

With the exception of Bukoba District and the western half of Ukerewe Island, ticks were more frequently found in huts containing fowls. In Bukoba, Ngara, Biharamulo and Geita Districts, and on Ukerewe Island, there was no difference in the observed abundance of the ticks in the presence or absence of fowls, but in all other areas, in huts containing fowls, ticks were nearly twice as numerous.

Prior to these observations it had been considered that these variations in tick behaviour were associated with high altitudes. In Ukerewe District, these variations occurred in the horizontal plane and, since the mean temperature of the tick habitat remained constant in both areas of variation and the mean relative humidity was 75 per cent. in the one and 83 per cent. in the other, it was now almost certain that the alteration in tick behaviour in relation to fowls was directly associated with humidity and only indirectly with altitude.

Tick infestation in the Lake Province, therefore, basically resembles that found in Kenya. In Kenya, the infestation was highest in the hot lowlands in the extreme south-east, and lowest in the cool, wet highlands in the Central Province, but the distribution was very discontinuous. In the Lake Province, infestations are least in the cooler, wetter country to the north-west, and increase in a south-easterly direction as the climate becomes progressively drier and warmer, and humidity becomes unreliable and erratic. The distribution of the tick is continuous, and only broken by the intrusion of the south-west corner of Lake Victoria.

The distribution of relapsing fever.—It has already been mentioned that when the incidence of relapsing fever in the highlands of Kenya is used as a standard of comparison, there is a wide discrepancy between the observed and expected incidence in Tanganyika. Roughly half of the relapsing fever recorded in Tanganyika Territory comes from the Lake Province where the discrepancy between the

TABLE VII.

Incidence of relapsing fever in the Districts of the Lake Province for the year 1952, extracted from the records of hospitals and dispensaries during the present investigation.

District	Recorded cases	District	Recorded cases
Ngara	42	Ukerewe	410
Bukoba	150+	Mwanza	235
Biharamulo ..	150	Kwimba	111
Geita	205	Maswa	127
N. Mara	80	Shinyanga	463
Musoma	100	Western Province	
Ukara Island ..	21	Kahama (1953)	162

observed and expected incidence is smaller than it is in the remainder of the Territory (Table II). In an attempt to find an explanation, an enquiry was made during these investigations into local medical opinion and records of cases of relapsing fever were collected from every traceable source for the years 1951 and

1952. Most of the case records are rudimentary and, although those from Bukoba Government Hospital have not been forthcoming (the total was 143 in 1953), the resulting total for the year 1952 was 2,094 (Table VII) compared with the 1,530 of the official annual return of infectious diseases. The discrepancy cannot, therefore, be accounted for by this enquiry. The difference is within the bounds of annual variation.

The incidence of relapsing fever found in the districts during these enquiries are given in Table VII.

Very few of the records provide details of individual cases of relapsing fever, but some are available from hospitals in Mwanza and Ukerewe Districts and give some useful information. For instance, in Mwanza town 63 per cent. of the cases are Africans who are complete strangers to the area. Since 1950, the native quarters in Mwanza have been treated with BHC dust and although tick infestations still remain, they have been drastically reduced. In consequence, 69 per cent. of all cases recorded in the District came from the rural areas. Active transmission

TABLE VIII.

Comparison of the recorded incidence of relapsing fever in infants, children and adults in urban and rural conditions in Mwanza and Ukerewe Districts.

	Incidence by age-groups					
	Number of cases			As percentage of total		
	Infants	1-16	Adults	Infants	1-16	Adults
Rural Mwanza	20	40	6	30	61	9
Urban African strangers	6	26	110	4	18	78
Urban indigenous Africans ..	10	37	43	11	41	48
Rural Ukerewe (eastern part) ..	20	61	84	12	37	51

Data for rural Mwanza from Sumve Mission and for Mwanza town from the Government Native Hospital in Mwanza; those for rural Ukerewe from Kagonguli Mission. The data from Nansio Town on Ukerewe Island are excluded since this small town contains a great mixture of non-indigenous tribes.

of the disease obviously takes place in the homes of the peasantry. The data given in Table VIII show that visitors to the town suffer heavily from relapsing fever, and it is probably this factor that has been, and still remains, the cause of the very high incidence of relapsing fever in towns in Tanganyika.

The Food of *O. moubata*.

Altogether 1,540 ticks were picked out as a haphazard sample from pooled catches and the blood-meal subjected to the precipitin test by Mr. B. Weitz at the Lister Institute in London. Only 30 of these samples were taken from ticks deliberately selected from specific sites.

At the start of the investigations, blood was taken from the stomachs of the ticks only when it was bright red. Very few ticks were found in this class and consequently, towards the end of the survey of Nyeri District, samples were taken from ticks containing dark-red blood in order to increase the size of the sample and this practice was continued in Taita and Digo Districts. As only nine negative results occurred among the first 293 samples tested, it was obvious that identification was possible long after a tick had fed (adult ticks do not normally

feed less frequently than once a month). This information was followed up by Weitz & Buxton (1953). In experiments carried out in London it was shown, when a group of ticks were fed on pig or fowl, that the stomach contents could be identified with an accuracy of 60 per cent. when tested between the 90th and 210th day following the test feed. In 64 ticks, variously fed on pig, fowl or monkey, the stomach contents could be identified in 97 per cent. up to the 65th day following a feed.

These results indicated that it would be well worth while sampling the stomach contents of ticks that appeared to be starved, and this was done during subsequent surveys when engorged ticks were scarce. This practice may have increased the proportion of negative results among the tests made on samples collected in the Lake Province, although Mr. Weitz, by applying various ingenious alternative testings, was able to show that some of these could be attributed to some kind of mammal. For example, while testing all samples for the presence of blood of man, domestic fowl, sheep or goat, and dog, he further tested a series of samples obtained from huts in Sukumaland against pig, ox, horse, cat and "general mammal", and in another series those positive to "general mammal" against rat. Although no sample was positive to rat in Sukumaland, there were positives from Ukara Island. All samples from Geita, Biharamulo, Ngara and Bukoba and a short series from Sukumaland were also tested for "general avian blood". These were positive only on four occasions and almost certainly were feeds made on domestic pigeons. These tests showed that all over the western part of the Lake Province of Tanganyika and to a lesser extent in Sukumaland, ticks were occasionally feeding on some unidentified mammal that was not always rat, but sometimes might have been rat, cat or possibly some nocturnal intruder such as mongoose or genet, but was most probably bat. There remained 200 negative results and it is possible that some of these might have reacted to snake or lizard had they been tested against reptilian antisera.

It has been observed that ticks will feed on one another and thus it would be possible to carry out a test on a blood-meal that had remained inside ticks for upwards to one year or even longer.

Seventeen samples were obtained from *O. moubata* collected in large burrows far out in the bush. Two from the Usambara area were tested against pig and both were positive. Fifteen from Shinyanga were tested against porcupine, pig and dog, and of 13 positive results all were pig.

Of 1,600 results from tests carried out on blood taken from *O. moubata* collected in African dwellings, 200 were negative, 52 reacted to "general mammal", 4 to "general avian", 6 to rat, 1 to cow and 1 to pig (the last three groups all from Ukara Island). The remaining 1,336 feeds are comparable, having all been tested against man (857 or 64 per cent.), sheep or goat (32 or 2 per cent.), fowl (416 or 31 per cent.) and dog (31 or 2 per cent.). These results show that the tick has an overwhelming preference for man or fowl.

It is quite obvious that *O. moubata* is very selective in its choice of food. Goats and sheep were present in a high proportion of the huts from which these samples were obtained and were numerous in infested huts during the night. Moreover, the people frequently sleep on the floor among the animals (there was only one bed to every 3 persons on an average in the Lake Province of Tanganyika). In many huts, it would be impossible for the ticks to feed on man without getting into close contact with the animals.

The proportion of feeds made by *O. moubata* on man, fowls, sheep or goats, and dogs is recorded in Table IX. These results show that in certain areas a high degree of selectivity is exercised when it comes to the choice between fowl and man.

In Table IX the feeds have been separated into three climatic groups. The cool and wet habitat group stands out conspicuously. It is clear that ticks living

in these areas do not feed on fowls. In all warm and hot country the ticks feed both on man and fowls, and the proportion feeding on fowls increases in the hot country. In Digo, they show a 93 per cent. choice for fowls. When it is considered that the chance of a tick taking more than one feed on the same type of host is high, the number of actual feeds made on man in Digo District fades into insignificance.

TABLE IX.

The composition of recognisable feeds made by *O. moubata* in African huts.

	Man	Fowl	Sheep or goat	Dog
In cool and wet habitats				
Nyeri	36	0	1	1
Taita	87	1	3	1
*West Lake	62	3	2	0
Total	185	4	6	2
%	94	2	3	1
In warm and moist habitats				
Sukumaland	170	73	9	10
Usambara	213	72	2	8
Ukerewe	193	36	9	2
Ukara	45	6	2	1
Total	621	187	22	21
%	73	22	3	3
In hot and moist habitats				
Digo	10	140	1	0
Mwakijembe & Daluni	42	85	3	8
Total	52	225	4	8
%	18	78	1	3

* West Lake includes the districts of Bukoba, Ngara, Biharamulo and Geita in Tanganyika.

In the warm habitat group the ratio of feeds on man and fowl is the reverse of that in the hot habitat. This is obviously a point of considerable biological significance and a possible explanation will be offered in the next section.

The conclusions reached in the section on Digo District to the effect that the ticks were not biting or infecting the human population is now confirmed. It has been established as a fact that very heavy infestations of *O. moubata* can occur in the closest proximity to man, and relapsing fever be non-existent. Can ticks be infected under these circumstances? The answer appears to be in the affirmative. Two hundred ticks from Digo were tested in the laboratory by inoculating each of 20 rats with the emulsified contents of batches of ten ticks. Three batches were positive. The estimated infective rate was approximately 1 per cent. (Walton, 1955). It cannot be said that this rate is either higher or lower than it was in other areas similarly tested, but it can be said that ticks from all areas so far tested, during these investigations, by this method, show a

very low infective rate, always less than 2 per cent. It is seen that when 94 per cent. of the ticks feed on man in the cold and wet habitats of Kenya (Table X), the incidence of relapsing fever in the presence of 3 ticks per hut was 0.82 per 1.000 per annum (Table II), and it will be realised that transmission of the disease at the best of times is in a precarious state of balance. A slight reduction in the abundance of ticks, or the deviation of a fraction of the bites to some source of food other than man, could upset this delicate balance.

Here is the obvious explanation of the discrepancies noted in Tanganyika between the expected and recorded incidence of relapsing fever. It can now be assumed that, if the ticks do not bite man in Digo District in Kenya, they will behave in the same way in hot country in Tanganyika, outside the sphere of our present investigations.

It is assumed that the explanation for the infection in ticks at Digo is that while they bite man sufficiently frequently to acquire infection, they do not bite man sufficiently frequently to complete the cycle. It is known that a tick probably does not normally feed much more often than 14 times during its maximum life-span, and obvious that the average tick in normal conditions will feed much less frequently. That these ticks can be infected is a fact; that they can transmit the disease is inferred from the occurrence of the disease in towns in central Tanganyika, possibly because fowls may be scarce, and the ticks are forced to bite man in the absence of their natural food.

A definite pattern of biological variation in *O. moubata* in East Africa now begins to emerge. In the north and west of Kenya and Tanganyika, and in isolated mountain peaks, in cool, wet country, ticks that feed exclusively on man occur in habitats with similar microclimates in which the temperature is 71°F. and the R.H. 86 per cent. Although ticks are not numerous, the incidence of relapsing fever is relatively high. When fowls are present the abundance of the ticks is uninfluenced.

In the extreme south-east of Kenya, and presumably in much of Tanganyika, ticks occur in hot country; they normally feed exclusively on fowls, and live in a different microclimate in which the temperature is higher and the humidity may be lower. These ticks are most frequently found, and are most numerous, in huts containing fowls. Relapsing fever in the presence of these ticks has a relatively low incidence and may be absent altogether.

The Domestic Fowl and its Significance in the Biology of *O. moubata*.

Phipps (1950), after a wide survey, wrote of the distribution of *O. moubata* in Tanganyika—"It is a fact that ticks are very often found in the earth where fowls habitually squat, and . . . it seems very probable that ticks which are present in the house tend to congregate where there are fowls, possibly because of the warmth." He stated that no relation existed between the presence of ticks and the presence of fowls, and based this assumption on statistical analysis of his results.

Those observations were based on a standard sampling method which involved the digging up of 4 sq. ft. of hut floor and passing it through a sieve. Apparently, he did not sample the walls. Our own data is based on a thorough search of the whole hut by a team of four to five, with the object of making the maximum possible catch obtainable. Each searcher was provided with a wide-mouthed pot and a hard wood probing stick used for locating pockets of loose earth in the floor and for tearing open holes and cracks in the wall plaster.

The data obtained by Phipps are compared, in Table X, with our data in the four areas sampled by both surveys.

Excepting Musoma, where agreement is very close, our data show a greater abundance. But even our figures must fall very far short of actuality for reasons given below. Our data for Kenya and the Usambara area can be regarded as far

closer to actuality than any data derived by us in the Lake Province of Tanganyika, for the simple reason that ticks are far more accessible in the former areas.

In the Kenya highlands, ticks are most uncommon in the walls of huts; among 450 infested huts examined, there was only a single instance. On that occasion, the ticks were found in the floor round the nest of a fowl and in irregularities in the wall up to two ft. above the nest in a small, warm hut high up in the Taita Hills. Ticks were also present in a neighbouring hut, and the housewife stated, and this seems now to be highly significant, that her husband

TABLE X.

Comparison of rates of infestation by *O. moubata* obtained by different methods by two investigators in four similar areas.

	Phipps		Walton	
	% huts infested	Mean no. ticks per hut	% huts infested	Mean no. ticks per hut
Kahama	72	11.9	80	28
Mwanza	43	5.5	75	15
Shinyanga	56	8.4	63	17
Musoma	6.6	0.7	5.5	0.6

Mean number of ticks per total huts examined.

worked at Taveta, near the Tanganyika border, where *O. moubata* occurs in hot, dry country (Heisch, 1950). It is an established fact that ticks are frequently carried over long distances in clothes, in blankets, and in boxes of personal effects.

In Digo District, ticks were uncommon in the walls; the plaster is often well-finished and most holes are found at the junction of the floor with the wall, and in the floor itself, but in all parts of Tanganyika visited by us, *O. moubata* was found in walls and in some huts it was difficult to find them elsewhere. Generally, in warm country, temperatures in walls are higher and humidities lower than they are in the floor. The kind of microclimate variation likely to be encountered may be seen by a comparison of the temperature and humidity differences in cracks in walls and in the floor at the base of the wall in huts where ticks were most numerous in the wall. In the cool country of the Usambara Mountains the temperature and relative humidity at the base of the wall was 67°F. and 93 per cent., respectively, and 2 ft. up the wall 69°F. and 77 per cent. The corresponding figures for the warm country of Shinyanga were for the base 72°F. and 55 per cent. and for 2 ft. up the wall, 76°F. and 50 per cent., respectively.

In cool country, the walls and the floor near its base are cooler than the remainder of the floor and there is a downward gradient in temperature and humidity outwards from the cooking fire.

Ticks were not often found in the walls of huts in the cool, damp country in the Usambara Mountains and they were found in a fowl's nest on only two occasions in the whole area of Usambara and the district west of Lake Victoria. In Sukumaland and Ukerewe, they were frequently found in the walls, particularly when in association with fowls. The ticks concentrate in the wall above the

fowls' squatting places, or occur, as many as fifty packed together in single dust-filled holes, at some distance from the fowls. These ticks advertise their presence by a spattering of white excretal marks on the walls and rafters. These marks are especially numerous round the entrances of long-frequented holes, and there seems to be no doubt that through constant use the ticks enlarge them, and they are usually filled with cast skins and egg shells.

On numerous occasions hard clay walls were deeply fissured and riddled with holes on the inside although outwardly appearing to be in a fair state of repair. Inside, the wood or grass stems that formed the original framework to retain the wet clay during building had been eaten away by termites and ticks had penetrated deeply into the resulting ramifications.

A standard sample of only 4 sq. ft. of floor selected from an average hut-floor space of some 380 sq. ft. as used by Phipps would obviously not include many of these concentrations of ticks.

In the endemic foci in the Kenya highlands, white excretal marks were extremely rare. The impression gained is that the ticks met with in the cool, wet highlands are sluggish and confine their activities largely to the floors of huts, while in the warmer and hotter areas they are active and run about the walls and rafters, and up into the thatch.

The mean air temperature in the high-altitude huts in day-time was 74°F. (fig. 7) with a tendency to rise slightly at night. This is the same temperature as that found in the tick microhabitat over a wide area of the Lake Province in Tanganyika where the night-time air temperature tends to fall below the day-time temperature. It is, therefore, somewhat difficult to see how temperature might be responsible for the difference in behaviour of the ticks in the two types of environment.

Working on a BHC Tick-eradication Scheme in Morogoro, Knowles & Terry (1950) stated "In most houses infestation was approximately the same in all rooms but areas where fowls and other domestic animals were kept, and especially their night resting-places, tend to be focal points of infestation, and ticks were found by the hundred on hens themselves", and they continue, "Such reinfestation as did occur was proven time and again to be due to fowls, which seem to be the chief hindrance to the total eradication of ticks in dwelling-places."

Ordman (1941), investigating relapsing fever in the Union of South Africa, states of *O. moubata* in the northern Transvaal, "relatively few ticks could be collected in the huts, but numerous typical punched-out holes were seen in the walls, and tick casts and secretion smears were abundantly in evidence", and then remarked, "In addition to *O. moubata*, the fowl tick *Argas persicus* was present." This clearly indicates that fowls were present in the huts in that area, and presumably the population of *O. moubata* fed on them. Ordman also noted that the ticks dropped from the roof during the night on to sleeping people.

Bedford (1924) describes *O. moubata* as being an important pest of fowls in South Africa, and remarks, "Should the ticks be found in the crevices of the fowl-houses, these should be treated in the same way as recommended for the fowl tick" (*Argas persicus* (Oken)). This clearly infers that *O. moubata* can subsist in South Africa on the blood of fowls alone.

From these observations, it is deduced that the ticks are associated with fowls and have a similar pattern of behaviour far to the south of the area of our investigations. Apart from occasional occurrences, this association reaches its northern limit along a line drawn between the Usambara Mountains and the Taita Hills in the east, across to Musoma on Lake Victoria, round the north of Ukerewe Island, and then straight south-west towards Kibondo and to Kigoma on Lake Tanganyika.

The difference in behaviour of ticks in the Taita Hills and in the Usambara Mountains can be stressed. The huts in the Taita Hills are small (the average

diameter being only 13 ft.) and fowls were found in 54 per cent. of them. Men, fowls, goats and sheep, dogs, cats and rats come into close proximity at night in this confined space. The ticks feed only on the human occupants and are most numerous in the damp higher altitudes. Infestation fades out towards the hot, dry country in the foothills. In the Usambara Mountain area where 70 per cent. of the huts contain fowls, the ticks were abundant at all altitudes, and the greatest concentrations were found in huts containing fowls. One quarter of the blood-meals tested were positive to fowl.

It would seem that in the Usambara area, an additional population of ticks was present, occupying the hot lowlands and spreading up into the high altitudes where it finds an abundance of fowls on which to feed. This second population seems to be almost entirely absent in the Taita Hills. One tick collected from a village in the hot foothills had fed on fowl, and ticks were found once at a high altitude associated with a fowl.

In the higher, wetter western end of the Usambara Mountain plateau, 83 per cent. of the tick feeds were positive to man, and this fell to 74 per cent. in the slightly lower eastern end. At Daluni Hill, to the east, it had fallen to 41 per cent. and at Mwakijembe, farther out on the hot plains, to 30 per cent. In the main Usambara Mountain bloc 50 per cent. of 131 positive blood-meal tests from altitudes below 4,000 ft. were positive to fowl, and this was reduced above 4,000 ft. to 22 per cent. in 193 similar tests. A similar gradation takes place even on the small mountain at Daluni, among samples of 20-28 tests in three categories. At 1,200 ft. on the plains near the mountain, 88 per cent. fed on fowl, at the base of the mountain 61 per cent. and at 2,700 ft. 40 per cent.

One gets the impression of a wave of ticks from the hot country in the south, washing northwards over the Usambara area up into Digo, but merely lapping against the Taita Hills.

In Ukerewe District, this phenomenon is repeated in the absence of altitude effects. The western extremity of the island receives a heavy rainfall which declines towards the east. In that wet area, 95 per cent. of the positive blood-meals reacted to human antisera although fowls were present in 44 per cent. of the huts. These ticks were, however, about four times as numerous as they are in the Kenya highland endemic foci.

One possible effect of the presence of fowls remains to be explained. Whereas the human population in a hut is fairly constant, the fowl population is not. The fowl population is fluctuating; it changes with the seasons, and with the inclinations of the owner. A hut may contain a dozen fowls one day and none the next; but the question is then whether the ticks turn to man as a source of food. The available data not only suggest that they can do so, but that their numbers dwindle to a low level. If this is true, it will be an important biological point of difference for, in the wet, cool regions of East Africa the tick population remained unaffected by changes in the abundance of fowls.

We do not know to what extent the population of fowls fluctuated in Mwanza and Ukerewe Districts. It is, however, a reasonable supposition that the people in Mwanza rural areas breed fowls for the market in Mwanza town, while Ukerewe is remote, and communications difficult, and that therefore the fluctuations are greater in Mwanza. This would imply that ticks in Mwanza fed alternatively on man or fowls to a greater extent, and formed a greater potential risk to the human population than they did in Ukerewe. Whether this is true or not, the data given in Table VIII suggest that the level of endemicity of relapsing fever was higher in Mwanza District than in the eastern part of Ukerewe.

There follows a summation of the data obtained during these investigations relating to fowls which in some ways carry these considerations a stage further.

According to our data, fowls were more numerous in hot country than they

were in cool country (Table XI) but it has not yet been ascertained whether this holds true in other parts of Africa.

Observations made in Digo District suggest that the tick may not occur in hot country if the rainfall is over 50 in. per annum even when fowls are present in considerable numbers. It is also worth noting that ticks were very scarce in the small available sample of huts that tended to be dry whereas they were five times as numerous in the surrounding damp huts.

TABLE XI.

Summation of data on the relationship between the incidence of *O. moubata* and the presence or absence of fowls in huts in three climatic groups.

	Cool & wet (mean microclimate temperature 70° F.)		Warm & moist (mean microclimate temperature 75° F.)		Hot & moist (mean microclimate temperature 80° F.)	
Total number of huts	2826		835		156	
	Fowls		Fowls		Fowls	
	Present	Absent	Present	Absent	Present	Absent
No. of huts	844	1982	444	391	119	37
Total no. ticks	3138	7704	9260	3651	2684	158
No. tick-infested huts	184	388	270	194	85	14
% of total huts	30	70	53	47	76	24
% of tick-infested huts	22	20	61	50	71	38
Mean no. ticks per hut	4	4	21	9	23	4
Mean no. ticks per infested hut ..	17	20	34	19	32	11

The variation in the proportion of ticks that fed on man and fowl in three broad climatic regions was given in Table IX. On passing from the hot to the warm climate there was a reversal in the feeding habits of the tick, and, in the warm climate, man replaced fowl as the main source of food. The data in Table XI show that the infestation rate was uninfluenced by the presence of fowls in the cool, wet climate, but was increased significantly in the presence of fowls in the hot climate. This difference is not so marked in the warm climate.

In the absence of fowls, ticks were no more abundant in the hot country than they were in the cool, wet country. But when fowls were present there was a great increase in abundance in the warm and hot country (Table XI).

No such change is seen in the cool, wet country. The increased abundance is related to the presence of fowls and is common to the warm and hot country only.

Variations in the abundance of ticks in the infested huts is shown in Table XI. The quantity remained unaltered in the cool, wet country, irrespective of the presence or absence of fowls. In the presence of fowls in both warm and hot country there was a considerable increase, but the quantity of ticks decreases in the absence of fowls in the hot country.

A hypothetical set of requirements is suggested which could fulfil these conditions. Two biologically distinct forms of *O. moubata* would have to be visualised. The first of these would be purely a human parasite and normally live in huts in cool, wet country. While this form could exist in huts in warm,

moist country, it could not do so in hot, moist conditions. The second biological form would normally live in a hot, moist country where it would feed on domestic fowls. If fowls occurred in large numbers in warm, moist or cool, wet country, this form would be capable of colonising huts in both climatic types. This hypothesis implies a wide tolerance to variations in temperature and humidity in the form that feeds on fowls, and it also implies that the two forms occur together in certain circumstances.

Some Observations on *O. moubata* in Animal Burrows.

O. moubata was found near the entrance in the burrows of large burrowing animals and in hollow baobab trees (Walton, 1953; Heisch & Grainger, 1950), and very few occurred at depths greater than six or seven ft. In some burrows these ticks are very numerous and almost invariably present a starved appearance.

Ant-bears (*Orycteropus*) are spectacular burrowers and make large numbers of holes although they rarely sleep more than once in any one of them. Large quantities of earth are dug up to block the entrance while they sleep, and are dug away the following night. While this habit creates conditions unsuitable to tick infestation, the older holes made by the ant-bears are much used by wart-hogs, hyaenas, jackals and pythons as transient hiding and sleeping places.

Porcupines (*Hystrix*) construct residential burrows on a lavish scale. A short tunnel usually connects a single entrance to a large circular domed cavern with a slightly domed floor. From this cavern, which may be six ft. high and ten ft. in diameter, other tunnels radiate to depths of twenty yards or more. These tunnels end in sleeping cells, and small by-pass caverns occur along them.

Wart-hogs make use of all these burrows, and creep in backwards to sleep and shelter, usually lying just inside the entrance, and would thus seem to be the most convenient natural host for these ticks.

There can be no doubt that Africans do occasionally transport ticks between their huts and these burrows, since rather futile efforts are sometimes made to dig and smoke out porcupines. In Sukumaland, however, there exists a strongly sectarian group or clan of professional porcupine hunters, who make large excavations and crawl down the burrows to kill porcupines. What happens to the ticks that these people must occasionally carry back to their huts is at present quite unknown.

Only once have we found a gravid tick in a burrow. This was a large specimen 16 mm. long, 12 mm. broad and 6.5 mm. in dorso-ventral thickness. Individuals that give the impression that a blood-meal may have been recently taken are usually found in the loose earth on the floor. Those that appear to be starved can be found in the day-time, sometimes in hundreds, clinging to the roof just inside the burrow entrance and if a disturbance is caused, they become very active, drop to the floor and run about in a purposeful searching manner. When given the chance they cling strongly to one's fingers, and appear to be more difficult to brush off than the hut-haunting ticks.

In hollow, infested baobab trees, white excretal marks were seen on the bark, and as these trees were fairly frequently visited by Africans from the Laboratory at Old Shinyanga, Tanganyika, it was thought that the ticks could have been introduced to the trees in clothing. The Africans stated that although this was possible, it was their opinion that the ticks in the trees were a different kind, since they were very numerous when the trees were first visited many years ago, and had gradually decreased in numbers as a result of the disturbance caused by the sifting of debris in search of tsetse fly pupae. Round these trees the earth was much routed by pigs, and quills of porcupines were found nearby.

In the burrows, the reactions of these ticks to light appear to differ from those observed in ticks from huts. Ticks found in huts are always negatively phototropic. Those found in wart-hog burrows will, when hungry, remain waiting at

agreement between the distribution of the tick and that of relapsing fever had originally awakened suspicions that the cause of these differences might lie within the species itself. Without recourse to a hypothesis of biological variation it became useless to seek for vague explanations for these disagreements.

In its wider distribution in Africa, the tick is absent from very dry areas and also from very wet ones. This suggested that more limited extremes of climate likely to occur within human dwellings in these areas might be effective in preventing tick infestation. Between these extremes the tick occurred in conditions that still varied widely and this fact appears to have been used to support the conception of the catholicity of the tick in its relation to climatic variation. But that same wide distribution could equally well have been used to support the opposite tenet, and is so used to support it here. The recorded distribution of *O. moubata* in huts is shown in fig. 4. This figure has been slightly modified from Leeson (1952) and given a more liberal interpretation. The distribution of rainfall, as far as it is ascertainable, is superimposed. Two essentially different distributions are revealed. One lies across the continent from Lake Victoria and Lake Tanganyika to the Atlantic. The other extends down the south-eastern side of the continent from Tanganyika to the Union of South Africa. The western distribution receives a rainfall between 40 and 60 in. per annum, and most of it lies in the rainfall area of 50 to 60 in. The eastern distribution receives a rainfall of 20 to 40 in. and, while most of it lies in areas with 30 in., it occasionally extends along migration routes. In Ethiopia and the Somaliland area, ticks appear to be sporadic in both climatic groups. The present investigations cut across the junction of the two distributions and have sampled conditions in both. Within these distributions the tick is absent or unrecorded in large areas. As already suggested this may be due to African housekeeping methods, it may also be due to geological reasons, or possibly to an absence of domestic fowls. The type of climate prevailing outside a hut must affect the climate inside it, and this again must affect the climate of the tick microhabitat.

In a wet, cool climate the ground is wet beneath a hut, and the cooking fire tends to dry it out and to reduce daily and seasonal variations. But the fire only succeeds in doing this effectively in small well-built huts of about ten ft. diameter. The larger the diameter, the more external variations affect the interior. In warm and hot climates the roof protects the ground beneath it and water is constantly brought in, but neither the presence of the roof, nor the water, can compensate for the rate of evaporation if the rainfall is low. In cool, wet climates the African fails to eliminate the prevailing high humidity and low temperature (see fig. 5). In warm and hot climates he cannot effectively lower the temperature or reduce evaporation.

The presence of the hut, however, does greatly modify the extremes of diurnal and annual variation and some examples of the diurnal variation are given in fig. 6. Maximum variation of the R.H. was within a range of 1.5 per cent. in 24 hours in a hut at Tiwi, Digo District, Kenya. An exceptional variation was seen in a large hut built of grass near Bukoba, Tanganyika, in which the maximum variation was 6 per cent. notwithstanding the absence of the housewife and a resulting discontinuity in the normal cooking fire routine. Temperature varied by 3°F. at Tiwi to 12°F. at Mwakijembe in Tanga District. Such small variations as these, occurring some three in. from the walls and floors, must be considerably reduced in the earth, and in holes and in cracks in the walls. For example, the seasonal and diurnal variations in a dust-filled hole at the base of a wall in a hut at Tiwi must be deduced as being extremely slow and minute.

Over 500 coupled readings were made of the temperature and humidity of the tick microhabitat, and of the air in the huts, once only in any given hut, and roughly once in every eighth hut examined. The microhabitat readings were made at the point of maximum tick infestation and are, therefore, very selective.

For instance, in a hut in Meru District, with a diameter of 14 ft., temperature varied by 12°F. and humidity by 18 per cent. along a 7-ft. radius of the floor. In this hut only one reading would have been made within this range. The air readings were taken with a whirling hygrometer and corrections made for altitude. Fine clinical-type thermometers were used for taking temperatures in the earth

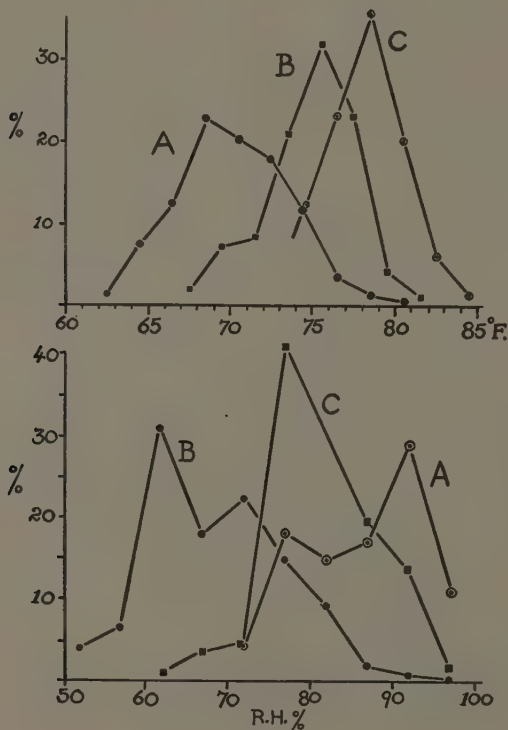


Fig. 5.—Examples of the distribution of the temperature (upper graph) and relative humidity (lower graph) of the tick microhabitat in huts in certain selected areas in East Africa (the number of occasions on which various grouped values occurred expressed as a percentage of total observations in each area). (A) In temperature distribution, Nyeri, Kenya (143 huts). In humidity distribution, Usambara Mountain plateau (190 huts). (B) Sukumaland (Mwanza, Kwimba, Maswa, Shinyanga & Kahama Districts) Tanganyika (96 huts). (C) Digo, Mwakijembe and Daluni, eastern Kenya and Tanganyika border area (66 huts).

and crevices. Humidity was measured with 1-cm. squares of an Electrolytic "A" Condenser Tissue impregnated with cobalt thiocyanate, using the method worked out by Solomon (1945). One of these papers was held in a notch at the end of a fine sliver of bamboo and could be withdrawn from a microhabitat and plunged into liquid paraffin without delay.

The effective range of these papers is from 60 to 100 per cent. R.H. In the drier areas of Tanganyika it would have been preferable had cobalt chloride been used instead of the thiocyanate. All our R.H. readings below 60 per cent. are unreliable, and those above 90 per cent. are subject to errors of two kinds. Firstly, at low temperatures, which usually coincide with these high humidities, the cobalt paper appears "redder" than it would do at the same humidity at

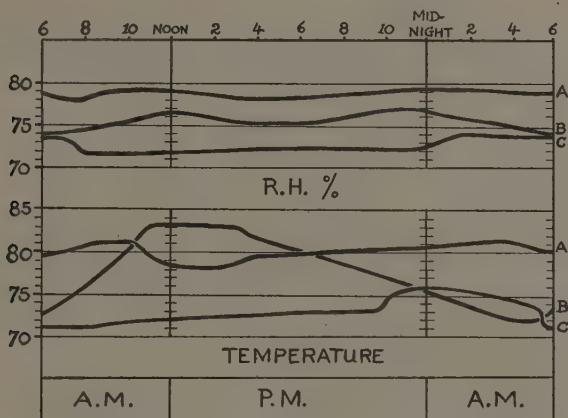


Fig. 6.—Examples of 24-hour thermohygrographic records made at a distance of 3 in. from tick-infested sites in huts in three selected East African localities. (A) Tiwi, Digo District, Kenya. (B) Mwajijembe, Tanga District, Tanganyika. (C) Bumbuli, E. Usambara Mountain plateau, Tanga District, Tanganyika.

higher temperature, and in areas of high humidity, where soils are frequently red, small particles sometimes cling to the paper and lead to errors on the high side. Secondly, patches of hut floor may have been wetted before our visit through numerous agencies such as a leaking roof, spilled water, rejected water from mouth- and hand-washings, ablutions, urination (animal and human) and through deliberate attempts to hide the presence of ticks by puddling. In wet climates, these patches dry out more slowly than they do in dry climates. Between 60 and 90 per cent. R.H., within which lies the range normally met with in the microhabitats, the readings may be regarded as very reliable and certainly within an accuracy of plus or minus 2 per cent. R.H.

While this data has revealed the type of climate with which *O. moubata* is normally associated, it has been further analysed in a search for additional evidence of biological variation. The data did not hold out much hope because the available range of variation was necessarily narrow. Temperatures below 64° and above 81°F. were rarely met with in tick microhabitats, and the size of workable samples is restricted to an effective range of little more than 15°F. The range of accurate estimation for R.H. is from 60 per cent. to about 92 per cent.

While impressions gained in the field may sometimes be misleading, and our impressions of high humidities are probably based on readings often 3 per cent. too high, it can, nevertheless, be stated that, generally, any large area in which the humidity of the microhabitat is consistently above 90 per cent. R.H. is free of tick infestation. Such conditions prevail in the higher, densely populated

altitudes in Embu District in Kenya, on Amani Mountain in the Usambara Mountain area, and near the coast in southern Digo District. The same principle applies to damp huts in areas otherwise tick-infested. But on numerous occasions it has been noticed that, in cool, wet country, gravid female ticks appear to seek out sites for oviposition that approach these apparently limiting factors. Why these female ticks choose to oviposit in damp earth, usually near the walls of huts, remains to be investigated. This habit contrasts with that of ticks living in huts in warm areas in Tanganyika, where ovipositing females were found in holes in the walls and roof posts more frequently than in the floors.

In the Kenya highlands, infestations were very uncommon at temperatures below 67°F. and in the Taita Hills practically none was found at temperatures above 78°F., or at humidities below about 74 per cent.

Similar generalisations are not so obvious in the case of the tick that feeds on fowls. Infestations occur at temperatures that range from 68° to 87°F. and the R.H. from about 50 per cent. to well over 90 per cent.

Some idea of the preferential R.H. is obtained, however, by a selected extraction of the data. The mean number of ticks per hut in the presence and absence of fowls in all huts examined in both warm and hot areas are given in Table XII after omitting all those huts containing large numbers of goats (numerous goats are sometimes inimical to successful tick infestation), and all huts occupied by members of the Jaluo tribe (Jaluo usually keep the walls and floors of their huts in a good state of repair and destroy vermin) in order to narrow down the number of variables. The results strongly suggest that, when fowls are present in the huts, the ticks are equally abundant in microhabitats at all values of R.H. except possibly the group with humidity greater than 84 per cent. But in the absence of fowls the ticks are most numerous in the dry microhabitats and least numerous in the wet ones.

TABLE XII.

A comparison of the abundance of *O. moubata* in the presence and absence of fowls in huts in relation to the humidity of the microhabitat.

R.H. %	Fowls present			Fowls absent			Mean no. ticks			
							Per total huts		Per infested huts	
	Total ticks	Total huts	Infested huts	Total ticks	Total huts	Infested huts	Fowls present	Fowls absent	Fowls present	Fowls absent
50-64	1260	27	25	390	12	9	47	32	50	43
65-74	911	19	18	469	20	18	48	23	48	26
75-84	1357	34	25	215	16	12	40	13	54	18
> 84	523	23	10	25	6	1	24	4	52	—

Somewhat similar data obtained in Ukerewe District give a very different result. Here, when fowls are absent, ticks are most numerous in the wet microhabitats and much less numerous in the dry ones (Table XIII).

Applying the hypothesis of biological variation, the data given in Table XII would imply that the "chicken-eating form" of *O. moubata* will be equally abundant at all values of R.H. likely to be met with in most African huts within the limits of the association previously outlined, as long as fowls are present in

a high proportion of the huts. In the absence of fowls, these ticks do not flourish at high humidities, but do flourish at low humidities of a value found commonly only in the drier parts of Sukumaland.

In the Ukerewe District of Tanganyika, temperature remains at a constant

TABLE XIII.

The effect of the presence and absence of domestic fowls upon the abundance of *O. moubata* in huts in three climatic regions * of Ukerewe District, Tanganyika.

	Fowls present				
	Total ticks	Total huts	Infested huts	Mean no. per hut	Mean no. per infested hut
Mainland	530	22	15	24	35
Island : east half ..	415	25	17	17	24
Island : west half ..	286	29	13	10	22
	Fowls absent				
	Total ticks	Total huts	Infested huts	Mean no. per hut	Mean no. per infested hut
Mainland	45	28	15	2	3
Island : east half ..	327	35	16	9	20
Island : west half ..	535	37	18	14	29

* The mean temperature is constant in all three regions. The mainland is dry, the island (east half) is intermediate and the island (west half) is wet.

level in the microhabitats in all three areas included in Table XIII. The variations in tick behaviour would, therefore, appear to be related only to humidity and, working from our hypothesis, the tick population in huts at the wet end of the island would be dominated by the "man-eating form". In this area the

TABLE XIV.

Mean R.H. % of tick microhabitat in relation to the proportion of ticks feeding on fowls in three climatic regions of Ukerewe District, Tanganyika.

	Precipitin tests			R.H. %	
	Total + feeds	No. positive to fowl	% positive to fowl	Total readings	Mean
Mainland	97	20	21	20	71
Island : east	60	12	20	16	78
Island : west	80	4	5	14	88

people occupy land that was, until quite recently, covered with tropical rain-forest; today, coffee is cultivated in the shelter of banana groves. According to Table XII, the "chicken-eating form" of *O. moubata* would be scarce in this area, possibly more scarce than is suggested since it is known that the data obtained at high humidities is biased on the high side. In the eastern part of the island, where date palms and pomegranates grow and the grass becomes withered, the "chicken-eating form" replaces the "man-eating form" and ticks are frequently found in the sleeping places of fowls and in their nests.

Nearer to, and in, the wet area, huts were found with heavy infestations in the absence of fowls, while others were uninfested in their presence. Ticks were found in parts of huts occupied by man, while other parts occupied by fowls were uninfested. Reference to Table XIV will show that the proportion of ticks feeding on man or fowl in these climatic areas would fully support the hypothesis.

The possibility that certain soils are inimical to O. moubata.

Reference is made to this subject under the heading of microclimate, since the existence of soils with inimical properties could provide an explanation for the absence of *O. moubata* from localities with apparently suitable microclimates.

During these investigations, three areas were found in which the soil appeared to be responsible for the sharp delineation of tick infestation: the hinterland of Digo District in Kenya, the southern portion of Nyeri District in Kenya where the land dips into the Fort Hall District, and Musoma and N. Mara Districts in Tanganyika.

In Musoma, the soil in the floors of huts contains quantities of coarse grit granules, although its geological derivation appears to be identical with infested soils to the south. In the other areas there is an actual change in the geological derivation of the soil. Tick infestation occurs in Nyeri, on Kikuyu Red Loam derived from Tertiary volcanics, and ceases abruptly on the fine sandy soil derived from Precambrian gneisses and schists. On Quaternary sediments and recent coastal deposits in Digo District, *O. moubata* was plentiful, but was absent in soil containing fine hard grit derived from Duruma sandstones and grits (Karoo). Van Saceghem (1923) also reported an absence of *O. moubata* from an area of certain volcanic derivatives contiguous to infested areas in the Belgian Congo.

Abrasion of *O. moubata* with alumina was shown by Lees (1947) to lead to a rapid loss of weight resulting from an enormously increased rate of evaporation through disruptions in the outer cement and wax layers of the cuticle.

That such effects may be present in nature is suggested by the practice adopted by some Africans in Geita and Kahama Districts in Tanganyika of incorporating a quantity of the dark grey "mbuga" soil in the mud plaster used in surfacing walls and floors. The absence of the tick from some huts in these heavily infested localities cannot be readily accounted for unless this practice is effective.

I am indebted to the late Dr. C. H. N. Jackson for making the suggestion that a village called Sayu near Shinyanga might repay a visit if there was to be any truth found in these assertions. Sayu is constructed of, and is built on, these "mbuga" soils, and although tick infestation is especially heavy in the surrounding areas, not a single tick could be found at Sayu.

However effective these soils may be in preventing successful colonisation by *O. moubata*, another more subtle factor may be involved. It will be seen, when the relation between the tick and relative humidity is considered more fully, that infestation by *O. moubata* is at its lowest in the presence of commonly occurring values of relative humidity approximately between 76 and 81 per cent. It cannot be mere coincidence that the mean R.H. of suitable tick microhabitats should lie between these non-productive values in all the areas in which the soils have been suspected of possessing abrasive properties. Conclusions are open to speculation, and must await future enquiry.

Temperature in relation to O. moubata.

Cunliffe (1921) concluded an investigation into the effects of temperature on *O. moubata* with the observation that it appeared to be a relatively unimportant factor. He showed that little difference occurred in mortality among developing groups of ticks kept at 30°C., which is just outside the normal range encountered in East Africa (fig. 7) and at 22°C.

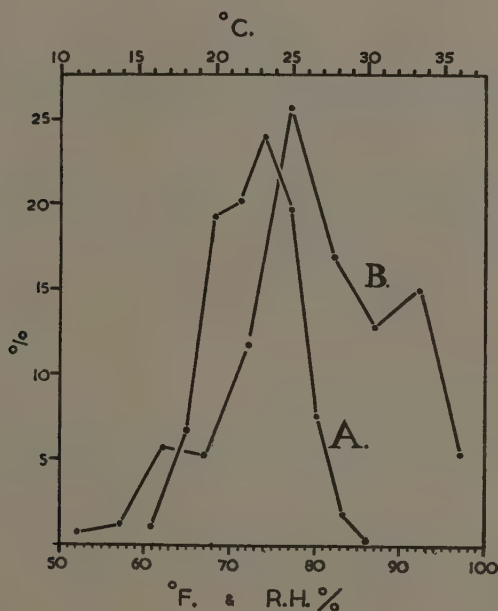


Fig. 7.—Distribution of temperature and relative humidity in "apparently suitable" tick microhabitats (infested and non-infested) in all East African huts examined during the present investigations. The number of occasions on which various grouped values occurred is expressed as a percentage of total observations. (A) Temperature (502 observations). (B) R.H. per cent. (567 observations).

The whole range of temperature variation of the microhabitat with which *O. moubata* is normally associated in huts or wart-hog burrows in East Africa is very restricted and somewhat precludes any hope of obtaining "critical" or optimum information from field data. Our observations on Ukerewe Island suggest that temperature has little influence on distribution, since it was a constant factor, allowing all differences in tick behaviour to be related to changes in the value of R.H.

As far as areas of infestation in the Kenya highlands are concerned, it could be said that the tick was rarely found in huts where the temperature of the microhabitat was consistently 67°F. or under, or 75°F. or over. Other parts of huts would generally be cooler, and very few of them warmer, than these extremes. There was a multitude of uninfested huts in which apparently suitable microhabitats occur with temperatures under 67°F. This figure may be critical in the conditions that form the environment in the African hut.

A summary of the data gathered during our investigations is given in Table XV. This shows a maximum incidence at 75 to 76°F., and probably reflects the proliferation of the "chicken-eating form" in Sukumaland in the presence of an abundance of domestic fowls. There is a risk of introducing distortions to the data if broken down into smaller samples through the inclusion of odd individual observations of high tick-catches. In only a few of the sub-areas visited is data

TABLE XV.

Mean number of ticks per hut in 2°F.-groups in infested huts among total observations made in East Africa.

Temperature of microhabitat (°F.)	64 65	66 67	68 69	70 71	72 73	74 75	76 77	78 79	80 81	82 83
Total ticks	188	644	2013	2157	3342	2389	2055	833	444	27
Infested huts	7	22	55	65	83	59	50	25	9	3
Mean no. ticks per infested hut ..	27	29	37	33	40	40	41	33	39	

sufficient to cover more than a range of 10°F. effectively, and when these are studied it becomes apparent that the peak of tick abundance corresponds with the peak of temperature distribution of the area concerned. For instance, the mean microhabitat temperature in Nyeri, Usambara, Meru and Taita, and all districts west of Lake Victoria was 71°F. At least in two areas maximum tick abundance occurs at the same value (see Table XVI).

TABLE XVI.

The abundance of *O. moubata* in relation to temperature of its microhabitat in African huts.

	In the Usambara Mountain plateau in Tanganyika						In the Nyeri District of Kenya					
Temperature (°F.)..	<67	67 68	69 70	71 72	73 74	75+	<67	67 68	69 70	71 72	73 74	75+
Total ticks	151	410	374	440	291	26	170	323	698	937	543	160
Total huts	10	20	13	11	10	8	22	28	34	31	21	16
Infested huts	5	11	10	8	6	2	8	11	17	19	11	14
Mean per total	15	20	29	40	29	3	8	11	20	30	26	10
Mean per infested hut	30	37	37	55	48	13	21	29	41	49	49	11

In Sukumaland, the mean temperature of the microhabitat was 75°F., and in Table XVII the peak of abundance is also seen to correspond with that value.

Ticks have been found in association with fowls at temperatures from 68° to 87°F., but it is impossible to gauge the range of the "chicken-eating form" in their absence owing to the small size of the available sample.

Temperature in the field affords unfruitful ground for investigation. It is impossible in the majority of cases to form an opinion of the effects of temperature in the presence of wider humidity variations which appear to be far more effective. Questions regarding the optimum and critical values of temperature requirements of the two hypothetical hut-haunting forms of *O. moubata* might be more readily answered in the laboratory.

TABLE XVII.

The abundance of *O. moubata* in relation to temperature of its microhabitat in African huts in Sukumaland, N. Kahama District and Ukerewe District in Tanganyika.

Temperature (°F.)	67-72	73-74	75-76	77-83
Total ticks	664	804	1463	842
Total huts	20	22	33	29
Infested huts	17	18	27	28
Mean per total	33	36	44	28
Mean per infested hut	39	45	54	30

Relative humidity in relation to O. moubata.

Some early observations on the effects of humidity on the developmental stages of *O. moubata* produced results that were startling in the degree of contradiction.

Brett (1939) found a 35 per cent. mortality in eggs of *O. moubata* at R.H. 30 per cent., a 57 per cent. mortality at R.H. 20 per cent., rising to 78 per cent. at R.H. 10 per cent. He gives evidence to support a conclusion that developmental stages up to and including the first nymphal instar were highly favoured by R.H. values of 80 per cent. and over, and that all lower values were progressively more unfavourable.

Cunliffe (1921) using somewhat crude experimental methods (possibly no more crude than the environment in which the tick survives in a high proportion of African huts) showed that a heavy mortality occurred in the early developmental stages in a "saturated" atmosphere; that no nymphs survived to reach the adult stage in a "moist" atmosphere, but a relatively higher proportion survived and some reached maturity in an atmosphere created by a layer of calcium chloride on the bottom of a jar.

There would be a temptation to regard such conflicting evidence with grave suspicion if it were not a fact that the behaviour of *O. moubata* under natural conditions in East Africa presents an anomalous picture only too reminiscent of the results obtained by these two workers.

Humidity is probably the foremost factor affecting the distribution of *O. moubata*; food, cover and temperature would appear to be subordinate. But no regularity is discernible among the multiplicity of conflicting evidence, unless the hypothesis of biological variation is introduced. Yet experimental evidence shows conclusively (Lees, 1946, 1947, 1948; Browning, 1954 *a* & *b*) that *O. moubata* is highly resistant to desiccation and is possessed of a remarkable ability to absorb water from the air at high humidities, and is thus able to remain at an almost constant weight for months without feeding. In the face of this evidence, and the general high level of humidity in which the tick is normally found in natural conditions, it is difficult to see how humidity could be of such

importance unless biological variation was present within the species and, at some stage during the life-cycle, humidity affected the varieties in different ways.

That relative humidity might affect *O. moubata* in its two hypothetical "forms" in this way, was suggested by data given in Tables I, XII and XIII,

TABLE XVIII.

Mean number of *O. moubata* per hut and per infested hut in R.H.%-groups among total observations made in East Africa.

R.H.%	< 62	62 65	66 69	70 72	73 75	76 78	79 81	82 84	85 87	88 90	91 93	94 96	97 99
Total ticks ..	808	912	1169	615	1363	2514	539	2152	1599	451	902	406	446
Total huts ..	20	26	34	15	46	147	29	75	60	24	60	21	19
Infested huts ..	17	24	20	13	32	78	16	55	32	12	28	10	10
Mean no. ticks per hut ..	40	35	34	41	30	17	19	29	27	19	15	19	23
Mean no. ticks per infested hut ..	48	38	59	47	43	32	34	39	50	38	32	41	45

and these suggestions are considerably strengthened by the data given in Table XVIII, and shown graphically in fig. 8. This shows the mean incidence of *O. moubata* in R.H. groups among the total readings taken in tick microhabitats during the present investigations. There are two distinct distributions with peaks of greatest abundance at 86 per cent. R.H. and again approximately at 67 to 68 per cent. At either end of the distribution there are marked distortions for which

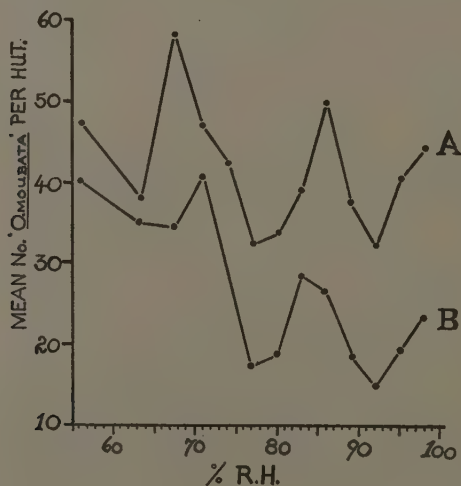


Fig. 8.—The abundance of *O. moubata* in huts with different values of relative humidity in the microhabitat. The mean number of individuals of *O. moubata* per infested hut (A) and per hut (B) in R.H.% groups among total observations made in East Africa (Table XVIII).

an explanation has already been given (p. 693). The point of lowest incidence corresponds with the largest sampled humidity group, that is, with values most frequently encountered in huts in East Africa (fig. 7).

There can be little doubt that these two peaks represent the distribution of the two hypothetical biological forms of *O. moubata* in their relation to humidity. They fit all the evidence that has already been elaborated. It is difficult to be certain of the exact position of the peak of abundance of the "chicken-eating form". There are high catches of ticks among the distorted lowest humidities which were probably estimated on the low side of actuality. There was no indication in the field that any diminution in tick abundance occurred in the dry southerly areas of Sukumaland investigated, where humidities were often obviously in the region of 50 per cent. R.H. At these values, cobalt thiocyanate papers fail to be effective. If it is considered that the rainfall in the southern portions of Shinyanga and northern portions of Kahama Districts lies between 19 and 30 in. per annum, and that the mean value of R.H. for tick-infested microhabitats in the middle of the dry season was 64 per cent., it is possible to surmise that it would not be very much lower than this value further south in areas of 15 to 25 in. of rainfall. It would seem unlikely that the "chicken-eating form" would be found in areas where R.H. values are consistently below 40 per cent.

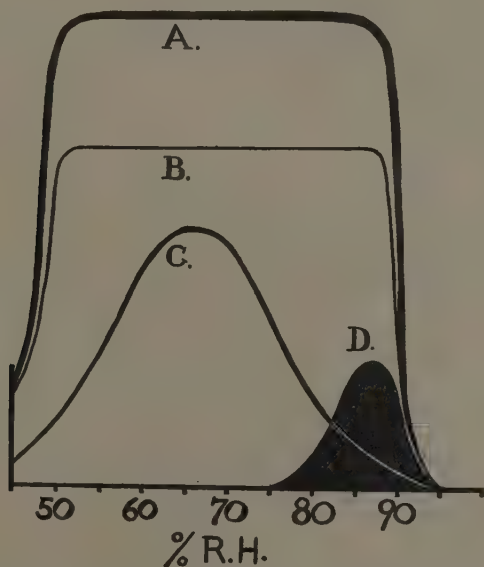


Fig. 9.—Hypothetical representation of the relative abundance of the "man-eating" and "chicken-eating" forms of *O. moubata* in huts with different values of microhabitat humidities. (A) "Chicken-eating form" in the presence of domestic fowls. (B) This represents the quantity of ticks obtained during a thorough search of a hut, the balance, shown in A above, remaining hidden in the walls of the huts. (C) "Chicken-eating form" in the absence of domestic fowls. The proportion obtained during a collection would be less, little more than shown for the "man-eating form" below. (D) "Man-eating form" in the presence and in the absence of fowls.

While some evidence exists of the presence of a further "form" of *O. moubata* in Africa, which is probably capable of living at R.H. values below 40 per cent., all the present evidence points to the fact that the hut-haunting "forms" under consideration are adapted to life in protected environments. In the case of the two hut-haunting forms, food procurement does not present any great hazards. On the other hand, the "wart-hog form" would be expected to differ from them by possessing characteristics enabling it to withstand occasional exposure when dropped in the bush, and to survive a serious hazard in the completely unpredictable nature of its food supply. Fig. 9 is a hypothetical interpretation of the data given in fig. 8 modified in the light of other evidence given in earlier sections. It illustrates the probable narrow limits of the distribution of the "man-eating form", compared with the wide distribution and greater abundance of the "chicken-eating form" in the presence of domestic fowls, and the probable reduced abundance of the "chicken-eating form" in the absence of fowls. It also shows the overlapping of the two forms. The "chicken-eating form" might masquerade as a "man-eating form" under certain conditions such as probably occur in Geita District in Tanganyika, where only 22 per cent. of the huts contain fowls in close proximity to country heavily infested with this form.

Saturation deficiency in relation to O. moubata.

Lees (1946) states "Water loss from the unfed tick is not closely related to saturation deficiency, particularly at high humidities. This departure is due to a physiological cause, namely, to the ability to secrete water", and in 1948 "One consequence of this physiological activity is a departure from Dalton's law at high humidities—for water-loss bears little relation to saturation deficiency".

These predictions appear to be borne out to a remarkable degree by our observations in the field in East Africa for, as shown in Table XIX, there is

TABLE XIX.

Mean number of ticks per hut, and per infested hut, grouped by huts with different values of saturation deficiency, in all huts examined in all areas investigated.

1/1,000th in. mercury ..	0 30	31 60	61 90	91 120	121 150	151 180	181 210	211 240	241 270	271 300	301 330	331 360	361 610
Total ticks ..	508	927	714	1127	1604	1852	1742	1621	381	564	736	418	566
Total huts ..	24	47	56	55	75	85	62	51	21	18	19	13	19
Infested huts ..	13	23	24	28	50	55	40	37	11	11	17	12	14
Mean no. ticks per total huts ..	21	20	13	20	21	22	28	32	18	31	39	32	30
Mean no. ticks per infested hut ..	39	40	30	40	32	33	43	44	35	51	43	35	40

practically no variation in the mean number of ticks occurring in microhabitats with a wide range of values of saturation deficiency.

A slight increase in abundance occurs towards the higher values instead of the reverse, which would not have been expected. This probably reflects, indirectly, nothing more than an increase in abundance of domestic fowls in drier areas. The incidence of *O. moubata* at different values of saturation deficiency for all warm and hot areas is given in Table XX. This suggests that if sufficient data had been available, a very different result might have been obtained if the data could have been extracted from huts in which fowls were absent. But, unfortunately, as so often happens when working in the field, the most interesting data are the most elusive, and as fowls are so prevalent in the huts, it would have been

necessary to estimate the incidence of ticks and of saturation deficit in 1,000 huts to obtain significant data. It could be deduced from Table XII that, as long as fowls are present, saturation deficit bears little relation to the abundance of ticks, but that if fowls were taken away, these ticks would be abundant only at the higher values. The reasons for this possibility are quite obscure. It is obvious that, although this tick probably can secrete water at

TABLE XX.

Mean number of ticks per hut and per infested hut in huts with various values of saturation deficiency in all warm and hot country investigated. Sukumaland, Ukerewe mainland, Digo, Mwakipembe and Daluni.

1/1,000th in. mercury	0-120	121-180	181-240	241-300	301-360	361-510
Total ticks	435	780	1620	614	1118	566
Total huts	25	25	55	27	26	16
Infested huts ..	8	18	38	16	26	14
Mean no. ticks per total huts ..	18	32	29	23	43	35
Mean no. ticks per infested hut ..	54	43	42	38	43	40

high humidities, such conditions can only occur occasionally, and consequently this mechanism might be highly efficient. In the dry intervals, the tick would obtain its moisture only from fowls. This suggests that its resistance to desiccation may be of a high order. Its scarcity at high humidities in the absence of fowls may possibly be due to entirely different causes related to developmental processes. These remarks, while being highly conjectural, are nevertheless worth recording, in view of the strange claims made by Cunliffe in 1921 (page 699).

TABLE XXI.

Mean number of ticks per hut and per infested hut in cool, wet country in huts with various values of saturation deficiency. Nyeri, Taita Hills and Usambara Mountain plateau.

1/1,000th in. of mercury	0-60	61-120	121-180	181-360
Total ticks	728	1323	2112	1099
Total huts	46	76	120	61
Infested huts	21	37	72	34
Mean no. ticks per total huts	16	17	18	18
Mean no. ticks per infested hut	35	36	29	32

The results shown in Table XXI for the "man-eating form" are exactly what would be expected following the observation of Lees quoted above. It should be mentioned, however, that this tick appears to be absent from areas

where the R.H. of the microhabitat is persistently above 90 per cent. The "equilibrium" humidity obtained by Lees was 92 per cent. At that humidity an unfed tick would lose weight very slowly indeed. If our high humidity estimation is biased on the high side, it would seem that this tick normally lives below the "equilibrium" value but would inevitably be exposed to such levels on occasions of heavy rainfall and could seek out such conditions if they were necessary to its survival. This tick would not be expected to withstand severe desiccation.

Experimental Evidence of Variation in *O. moubata*.

On returning to the laboratory after the visit to the Lake Province of Tanganyika, numerous cultures of *O. moubata* were available for study.

Small numbers of specimens of comparable size were selected by the assistants from a series of the cultures and were placed in pairs in numbered petri dishes of similar make, and examined macroscopically against a white background in an even source of daylight and in complete ignorance of their place of origin. The results of attempts to place these ticks into groups were only 50 per cent. successful and somewhat inconclusive.

All these ticks closely resemble one another when in either the starved or engorged state, but it was observed that as they settled down after taking their enormous blood-meals (which weigh up to six times their original body weight) they take on a certain recognisable and characteristic appearance which becomes increasingly apparent during the first week. The "man-eating", "chicken-eating" and "wart-hog" forms appear to concentrate the meal and lose weight at different rates and in that order. These tests were then repeated using ticks "standardised" by feeding them all on the same day, and examining them a week later.

The results of these tests were decidedly encouraging. Ticks from animal burrows were easily recognised from the start, and so were ticks representative of the Mount Kenya area. The remainder gave some difficulty. The tests were repeated on groups of males and groups of females, this time five in each group. The results were more conclusive than the first tests and also revealed the presence of individuals in some cultures from huts in Tanganyika that did not agree in appearance with the remainder. This never happened in groups representative of cultures from wart-hog burrows or huts in the Mount Kenya area.

The important point arising from these tests was that material of *O. moubata* from certain areas differed in appearance sufficiently to allow their repeated accurate recognition, and that the recognitions corresponded with expectation.

It was decided to select immediately specimens from cultures which showed the least variation between individuals, irrespective of any difficulty experienced in correctly guessing their origins, and subject these to a set of simple experiments that would underlay other more active investigations. These experiments were of three kinds, to test female productivity, length of life, and the effects of cross-breeding. It was decided to conduct these tests under conditions that, while being constant, would otherwise remain as natural as possible. The floors of small dishes were covered with a layer of soft earth over a layer of hard clay, following the work of Robinson (1942), and the ticks were placed in these, inside a desiccator in a constant R.H. of 85 per cent., and at a temperature of 74°F. All ticks were allowed to feed to repletion on the ear of a rabbit.

Only virgin male and female ticks of comparable ages were used. In the productivity experiments each male and female was fed before the initial oviposition and again after each oviposition. In the test for length of life the ticks were given one initial blood-meal only. This experiment included groups of segregated virgin females and segregated groups of virgin males, and also groups of both virgin males and virgin females mixed only at the commencement.

The cross-breeding experiments were started early in 1955 and are, at September 1956, far from complete. It has been established, however, that crosses of ticks from all localities can produce viable offspring, some of which have reached the adult state. Further results must be described elsewhere. Hybrid ticks could be expected to occur in certain areas where different forms overlap.

It will now be more readily understood why this paper contains no allusions to the subject of morphology. Limited taxonomic work has been carried out on ticks from wart-hog burrows from the Mount Kenya area and the Usambara Mountains. Morphological differences were observed in ticks from these sources, but the range of variation presented by the material from Usambara indicated that this is a subject of considerable complexity calling for protracted and detailed investigation.

Material used in the experiments on length of life and productivity was as follows:—

Group 1. "Wart-hog form."	Progeny of ticks obtained from a wart-hog or porcupine burrow on Crescent Island, Naivasha, Kenya. Altitude 6,260 ft.
Group 2. "Wart-hog form."	Progeny of ticks obtained from a wart-hog or porcupine burrow at Daluni, near the Usambara Mountains, Tanga District, Tanganyika. Altitude 1,000 ft.
Group 3. "Chicken-eating form."	Progeny of ticks obtained from an African house at Tiwi, one mile from the coast in Digo District, Kenya. Altitude <i>ca.</i> 50 ft.
Group 4. "Man-eating form."	Progeny of ticks obtained from a group of African huts in Wuchichi village at 5,000 ft. above sea level in the Taita Hills, Kenya.

The Naivasha "wart-hog" group was not included in the experiment on length of life.

Five male and five female ticks from each group took part in the productivity experiment. In the tests for length of life, groups 3 and 4 were represented by 40 individuals of each sex segregated, and 20 of each sex mixed. Insufficient material was available from group 2, and this was represented by 20 individuals of each sex segregated, and ten of each sex mixed.

Results of female productivity tests.

These are given in Table XXII. Statistically significant differences occur in the egg-production of the three forms in all comparisons but two. A similarity is shown only in the mean number of eggs per batch between the "chicken-eating form" and the combined groups of the "wart-hog form", and in the mean number of egg batches produced per female between the "man-eating form" and the "wart-hog form". In the two instances where similarity was expected to occur in two groups with origins separated by 300 miles and 5,000 ft. of altitude, it was proved to a remarkable degree, as shown below in the case of the two groups of the "wart-hog form" under test.

	Total eggs laid	Mean number of egg batches laid
Group 1	6610	8.6
Group 2	6697	9.0

The similarity allowed the groups to be joined in the significance tests.

Results of tests on length of life.

These observations commenced in the case of the "man-eating form" and "chicken-eating form" in July and August 1953, dated from the last moult of the isolated virgin females. In the case of the "wart-hog form" they commenced in November 1953. This experiment is not likely to be completed until 1958 but in September 1956 the position was that out of the original 300 ticks entering the tests, 25 per cent. remained alive. Of those selected from African huts, 92 per cent. had died and the survival was almost entirely confined to the "wart-hog form". Experiments of this kind take a long time to complete, but are very necessary to determine the reactions of these ticks under "natural conditions", in order to obtain some standard on which to base other experiments made under abnormal conditions, designed for quicker results and to accentuate any observed tendencies towards variation.

Under the conditions of the tests, little difference between the forms ("man-eating", "chicken-eating" and "wart-hog") has been noted in respect of the mortality rate of males in the absence of females, or of mixed lots of males and females. On the other hand, there was a difference between the "man-eating" and "chicken-eating" forms as regards rate of mortality of segregated, virgin females.

The rate of survival among the groups of virgin females of the three biological forms is shown graphically in fig. 10.

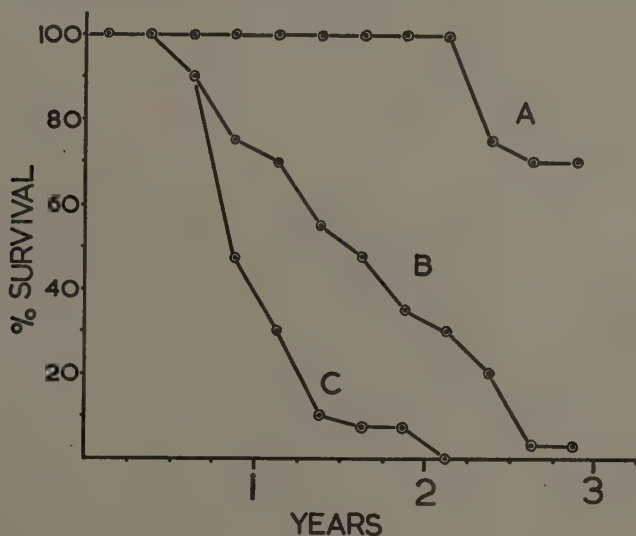


Fig. 10.—Survival of groups of virgin once-fed females of *O. moubata* kept at constant temperature and humidity. The groups were suspected of possessing biological differences and the humidity (85%) was chosen to favour the "man-eating form". This experiment continues. (A) "Wart-hog form". (B) "Chicken-eating form". (C) "Man-eating form".

The resistance to starvation displayed by the "wart-hog form" is astounding. But the isolated virgin females of the "chicken-eating form" survived nearly twice as long as the "man-eating form", and, most significantly, under conditions that could only be surmised as highly favourable to the latter.

Summary.

An investigation has been made into the distribution and bionomics of *Ornithodoros moubata* (Murr.) in East Africa in relation to the incidence of relapsing fever, and a survey has been made of the infestation in over 4,600 African huts, together with the temperature and relative humidity conditions.

In Kenya Colony, relapsing fever is endemic in the high rainfall areas of Meru, Nyeri and Taita Districts. These habitats are cool and wet with a mean microhabitat temperature of 71°F. and a relative humidity of 86 per cent. Tick infestations were relatively sparse and were rare in the hot and dry climate of Embu District, the base of the Taita Hills and generally over all such country in Kenya.

In Tanganyika Territory, relapsing fever is widespread, and the most striking difference was the relatively much greater abundance of the tick, especially in the dry central areas. It is pointed out that although relapsing fever is most prevalent in the north-west, endemicity is at a lower level than in Kenya, and decreases towards the south-east, indicating that the degree of incidence of the disease does not conform with that of the vector.

In the Digo District, south of Mombasa on the Kenya coast, ticks showed a reversal in their choice of microclimate from those in the cool highlands and were numerous in hot, moist conditions. The incidence of the disease was very low.

O. moubata was widespread in the Usambara Mountain area of Tanganyika. Ticks were most numerous in the cool, wet conditions above 4,000 ft., but were also abundant in the hot, moist foothills and plains, whereas they were absent in hot and dry country at the base of the Taita Hills in Kenya 80 miles to the north.

As humidity appeared to be a foremost factor affecting the distribution of *O. moubata* it was not possible to evaluate clearly the effects of temperature. It is suggested that all the conflicting evidence of the relationship of the tick populations to microclimate and the incidence of relapsing fever may be explained only by introducing a hypothesis of biological variation in the tick itself. It is shown that there are two peaks of greatest abundance, at relative humidities of 86 and 67 to 68 per cent., respectively, and it is suggested that these two peaks represent the distribution of two hypothetical hut-haunting biological forms.

An examination of the blood-meals from pooled catches by the precipitin test showed that in the cool and wet habitats of the Kenya highlands and the north-west of Tanganyika, 94 per cent. of the recognisable feeds were on man and only 2 per cent. on fowls. In the hot and moist habitats of Digo and the low-lying area between Digo and the Usambara Mountains, 18 per cent. were on man and 78 per cent. on fowl. In the mainly warm and moist habitats of the Usambara Mountains and the area bordering the south-east of Lake Victoria, 73 per cent. were on man and 22 per cent. on fowls.

It is therefore suggested that there are two biological forms of *O. moubata* found in huts, one feeding on man and the other feeding on fowls. The former is found in huts at high altitudes in areas having a cool and wet climate; it is essentially a human parasite showing a marked preference for the blood of man while ignoring the presence of fowls however numerous or available. It occurs in greatest abundance at a relative humidity of about 86 per cent. It is found at relatively low temperatures from 67° to 75°F. It is absent in areas where the microclimate is consistently over 90 per cent. R.H. and may not occur where it is consistently lower than about 74 per cent.

The form that feeds on fowls appears to possess a tolerance to a wide range of temperature and R.H., occurring in greatest abundance at 67 to 68 per cent. R.H. It is found at temperatures from 68° to 87°F. It is more resistant to starvation than the form that feeds on man.

Evidence is given indicating the existence of a third biological form, in the

burrows of wart-hogs and porcupines, that does not appear to be associated with African huts. This form is extremely resistant to starvation.

Experimental evidence is given that these three forms differ significantly from each other in their egg-production characteristics. They can also be identified macroscopically with a fair degree of accuracy but cannot at present be separated on a morphological basis.

From the evidence available, the distribution of the form that feeds on man is thought to include the southern half of the Belgian Congo, west to the Atlantic and east to Lake Victoria, with isolated pockets in highland areas scattered over East Africa to the Indian Ocean. The form that feeds on chickens extends from the southern shores of Lake Victoria to the Union of South Africa.

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THE MAIZE AND SORGHUM STALKBORER, *BUSSEOLA FUSCA*
(FULLER), IN PEASANT AGRICULTURE IN
TANGANYIKA TERRITORY.

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The Lepidopterous borer, *Busseola fusca* (Fuller), has been recognised as a major pest of maize and sorghum in all African countries south of the Sahara (Jepson, 1954). In Tanganyika it is without doubt the most important insect pest of these cereals and is responsible for serious loss in some part of the Territory every year.

The bulk of the maize and sorghum produced in the Territory is grown by African peasant farmers on small plots cultivated by the hoe. The main sowings are begun with the onset of the early rains in November or December and continued until the rains end, usually in April. Yields are variable, but in general low. Maize and sorghum plant residues are generally left standing in the fields after harvest until the next main sowings begin.

The investigations reported on in this paper were designed to obtain information on the biology of the insect in the above conditions and to devise a simple routine of control suited to the peasant farmer. Results are given of insecticide trials against *Busseola* on peasant-grown maize using 2.5 per cent. DDT dust.

Distribution in the Territory.

Busseola has been recorded at Handeni (altitude 2,200 ft.) and Newala (2,600 ft.), but is more particularly abundant in the Central Plateau area (4,000–5,000 ft.)

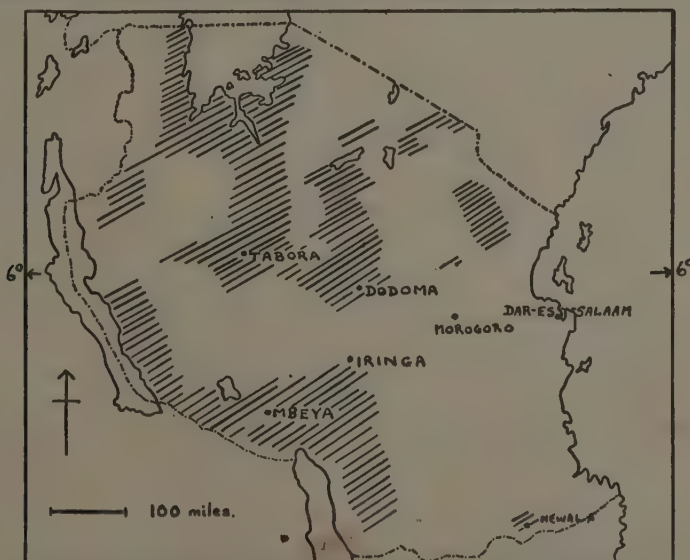


Fig. 1.—The known distribution of *Busseola fusca* (Fuller) in Tanganyika.

TABLE I.

The length of stages in the life-cycle of *Busseola fusca* (at av. temp. 26.4°C.).

Stage	Egg	Larval instars					Pupa	Adult (emergence to egg-laying)
		1	2	3	4	5	6	7
Duration of stage (days)	7.0 (6-9)	3.0 (2-5)	3.3 (2-4)	3.0 (2-5)	3.2 (2-5)	3.7 (2-5)	8.1 (4-11)	11.0 (9-14)
No. of individuals	38	38	35	34	29	23	18	4

Mean life-cycle (egg-laying to egg-laying): 51.8 days (43-55).
 Mean length of larval life: 28.3 " (24-36).

TABLE II.

The survival of full-grown larvae of *Busseola fusca* collected in the field at Mwanhala, Western Province, and kept in the laboratory without food or water.

Date	Days after collection	Live larvae present	Cumulative totals of		Remaining living larvae as % of original total	Pupae as % of original number of larvae
			Dead larvae	Pupae		
28.vi.54	0	405	0	0	100.0	0.0
1.vii.54	3	344	30	31	84.9	7.6
1.viii.54	34	307	52	46	75.8	11.4
1.ix.54	65	293	64	48	72.3	11.8
1.x.54	95	251*	71	63	62.0	15.5

* 20 larvae (4.9% of original total) missing.

and in the Northern and Southern Highlands farming areas which rise to about 9,000 ft. above sea level. It is absent from the coastal belt and from hinterland areas up to about 2,000 ft. (fig. 1). The main factor limiting the distribution of the insect would appear to be the higher temperature experienced at the lower altitudes. This view is supported by the observation that females bred out in the comparatively hot conditions of Morogoro (1,900 ft.) have frequently failed to produce viable eggs, although they were known to have been mated.

Laboratory Observations.

Breeding and life-history.

Pairs of newly emerged adults were placed in gauze-covered insect cages, with dimensions of 2 ft. \times 2 ft. \times 2 ft., the bottoms being covered with dry maize trash, or in gauze-covered 20-oz. bottles containing a small length of the terminal shoot of sorghum which served as a foothold and a place for egg-laying. In the cages, the eggs were laid indiscriminately on the gauze or in the trash; in the bottles, the eggs were usually laid beneath a leaf sheath as is the habit in the field. On hatching from the egg, each larva was transferred singly to a fresh piece of sorghum stem, cut from the upper part of a tiller or young plant, that had a whorl of developing leaves which served as food. The larvae were kept separately in gauze-covered 14-oz. bottles, inspected daily and transferred to fresh food as required. From the beginning of the third stage, the larvae were inserted into small cells scooped out of the side of more mature pieces of sorghum stem. After sexing, the pupae were placed singly in empty 14-oz. bottles, where the adults emerged. The breeding results are given in Table I.

Of nineteen individuals taken through from egg to pupa, one pupated after the fifth instar, fourteen after the sixth and four after the seventh; six larval instars would thus appear to be the normal number for *Busseola*.

Diapause.

Given adequate supplies of suitable food material it is possible to breed *Busseola* continuously throughout the year. However, under unfavourable conditions caused by the drying out of the food material, fully fed larvae go into a state of diapause, usually preceded by one or two moults. Once the larvae have entered this state it is possible to keep them alive without food or water for considerable lengths of time. The results of an experiment in which this was done are given in Table II. The larvae concerned, which had been collected in the field, were kept in the laboratory, each in a glass tube, the open end of which was closed with a plug made from a rolled piece of "windolite" which the larva could not chew and thereby escape. However, the plugs were lightly inserted, to allow the larvae to respire, and because of this some escapes did occur. It will be seen that, three months after collection, 62 per cent. of the larvae were still alive, and 15.5 per cent. had pupated.

The resumed development of the diapausing larvae can be initiated by bringing them into contact with water. In laboratory tests, pupation was brought about by placing the larvae in fresh food, in old stems soaked in water, and even in damp blotting-paper. Dipping larvae in water and subsequently keeping them in an atmosphere saturated with water vapour did not significantly induce pupation. The results of these attempts to induce pupation in diapause larvae are given in Table III. Once pupation had taken place, emergence of the adults followed in the usual 12-16 days obtaining under Morogoro conditions.

Field Biology.

Incidence in relation to sowing time of crop.

The main features of the field biology of *Busseola* in relation to the plant itself have been described by a number of authors (Jack, 1917; Mally, 1920;

TABLE III.
Induction of pupation in diapause larvae of *Busseola fusca*.

Days after beginning of experiment	Percentage of larvae undergoing pupation					
	Treatment					In dry stems (control)
	Immersed in water for 10 min., subsequently kept in damp atmosphere	Immersed in water for 20 min., subsequently kept in damp atmosphere	In damp atmosphere only	In stems kept damp by wetting twice daily	In damp blotting paper	
2	0.0	0.0	4.4	—	0.0	0.0
7	—	—	—	5.7	—	—
9	—	—	—	—	33.3	—
13	—	—	—	—	46.6	—
21	4.0	6.0	6.6	60.0		7.0
28	—	—	—	65.7		—
33	8.0	12.0	8.8			9.4
No. of larvae at beginning	50	50	45	35	15	85

Anderson, 1926). Eggs laid beneath the leaf sheath give rise to larvae which in young plants first feed on the upper surface of the leaves, causing a typical scarification by which their presence is recognised, and later penetrate the heart by way of the "funnel" of leaves. Crop loss is caused either by death or a reduction in the vigour of these young plants or by a reduced vigour in older plants as a result of larvae feeding in the stems.

Duerden (1953) concluded from work on mixed infestations of *Busseola* and *Chilo zonellus* (Swinh.) in maize and sorghum at Kongwa, Tanganyika, that such factors as time of planting and age of the plant appear to have little effect on the infestation. Where, however, *Busseola* is the main pest it is a matter of frequent observation that the incidence of damage to young plants is most definitely connected with the time of sowing in any one locality. From the investigations carried out, it appears that the first sowings, made within two to three weeks of the beginning of the rains, are likely to be heavily attacked by larvae of the first generation that are the progeny of a "rain-induced" flight of adults of *Busseola* derived from diapause larvae in the maize and sorghum stems left in the fields from the previous season. Later plantings largely escape until the time of emergence of the adults of the first generation, 10 to 12 weeks after those derived from the diapause larvae, when young plants are again heavily attacked. Wide variations in sowing date in African peasant agriculture are occasioned by the dependence of cultivation on the hoe. In many areas preparation of the land cannot be started until the soil has become sufficiently softened by the first rains in November or early December; in order to obtain an acreage sufficient for family requirements, cultivation and sowing must be carried on whenever adequate rainfall occurs throughout the period from November to April.

Number of generations in the field.

Busseola moths begin egg-laying on the first sowings of maize or sorghum when the plants are about two weeks old. The egg-laying period observed in the field is brief and well defined. In the Nzega district of Western Province, in the 1954-55 season, egg-laying on maize sown on 11th December 1954 began on 27th December and ended on 13th January 1955, a period of 18 days. At the earliest, sowings could have begun on 5th December 1954, as the first adequate rain of the season, 0.98 in., fell on 4th December 1954, so that the maximum spread-over of egg-laying could not have exceeded 23 days. In the previous season, the beginning of egg-laying was not recorded, but daily examinations of young plants from the beginning of January 1954 showed that no further eggs were laid by the diapause generation of moths after 19th January 1954.

In the 1954-55 season the first eggs hatched on 3rd January 1955, seven days after laying. In examinations made at weekly intervals, pupae of the first generation were found on 7th February 1955, giving a minimum larval period of 30-36 days; pupal exuviae were found on 7th March 1955, giving a minimum pupal period of 22-28 days. All larvae had pupated by 19th April 1955, 124 days after germination of the maize. Eggs of the second generation were found on 9th March 1955, giving a minimum period of 72 days for the complete life-cycle, egg to egg. This exceeds the figure (51.8 days) obtained from laboratory breeding at Morogoro, as is to be expected from the fact that Western Province temperatures are lower than those at Morogoro.

The beginning of pupation of the second-generation of larvae was not recorded but pupae were present, together with larvae, in both maize and sorghum stems collected at Tabora, Kahama and Nzega on 30th June 1955. The *Busseola* larvae in these maize and sorghum stems were mostly fully fed individuals of the second generation, but examination of sorghum tillers collected on the same date revealed large numbers of first, second and third larval stages of *Busseola* of the third generation. In subsequent examinations of maize and sorghum stems, collected

TABLE IV.

Pupation of diapause larvae of *Busseola fusca* during the early period of the rains in maize and sorghum stems left standing from the previous season, Tabora, 1954-55.

Date of sampling	No. of stems examined		No. of larvae present	No. of pupae present	Pupae as % of total	Average rainfall for month (in.)
	Maize	Sorghum				
30.vi.55	150	—	97	9	8.5	June 0.00
30.vi.55	—	150	239	3	1.2	July 0.00
31.viii.55	—	50	34	0	0	August 0.00
30.ix.55	100	—	19	0	0	September 0.42
30.ix.55	—	100	40	0	0	
31.x.55	150	—	41	0	0	October 0.47
31.x.55	—	150	64	1	1.5	
19.xi.54	50	—	104	14	11.9	November 1.84
19.xi.54	—	76	100	74	42.5	
6-11.xii.54	120	—	14	6	30.0	December 4.63
6-11.xii.54	—	400	519	149	22.4	
31.i.55	—	200	0	0	(116 pupal exuviae)	January 6.20

on 31st August, 30th September and 31st October 1955, only fully fed, or almost fully fed, larvae were found; pupae were absent. The fully fed larvae in these maize stems were undoubtedly diapause larvae of the second generation; the larvae in the sorghum stems must have been of both second and third generations, the second generation of larvae diapausing in the older sorghum stems and the third generation developing in the sorghum tillers, which had matured considerably by the time the examinations were made.

Termination of diapause in the field.

The observation that *Busseola* exists in the dry-season months from August to November mainly in the larval stage has been confirmed from a number of areas in the Territory. Examination of standing maize and sorghum stems shortly after the onset of the rains in November–December reveals an increase in the number of pupae in relation to larvae, whilst no live larvae or pupae have been found in such old stems as have been allowed to remain in the fields until the following January or February. In Table IV, which shows this increase in the number of pupae during the period of the early rains in Tabora, Western Province, the data for the months of June–October were obtained during 1955 as it was not possible to carry out examinations for those months during 1954.

These observations are in agreement with those of Evans (1939), for *Busseola* in South Africa, *i.e.*, that the pupation of larvae overwintering in maize is hastened by abundant winter and early spring rains. They also afford field confirmation of the laboratory experiments, described earlier, in which pupation of diapause larvae was induced by contact with water.

Alternate hosts.

No alternate hosts of importance comparable with sorghum and maize have been identified. Reliably identified larvae have been found in wild sorghums in the Lake Province. The record of Jepson (1954) of *Busseola* in elephant grass, *Pennisetum purpureum*, in Uganda indicates that the insect may also be present in that grass in Tanganyika; the only Noctuid found by the writer in elephant grass is *Sesamia calamistis* Hmps. In many of the drier areas where *Busseola* is a serious pest, elephant grass is not an important constituent of the flora. Of the food crops, bulrush millet, *Pennisetum typhoides*, has been found to be attacked by *Busseola* but very few larvae per stem are ever found.

Cultural Control.

As larvae of *Busseola* pass the dry season in the stems of maize and sorghum, destruction of these crop residues would seem to be the obvious method of control. This, in fact, was the method advocated by Duerden (1953) on the large acreages of maize and sorghum sown by the Overseas Food Corporation. In peasant agriculture, however, the dry stalks are often required for purposes such as palisading or the building of contour banks; in the cattle areas of Western and Lake Provinces the sorghum is deliberately left in the fields to tiller and provide some sort of grazing for the animals during the dry season. For these reasons a considerable proportion of crop residue inevitably remains in the field. Late sowing to avoid the diapause generation of moths emerging on the rains offers, in theory, a method of minimising *Busseola* damage. Owing to the unreliability of the rains and to the limited area which can be cultivated by hand at any one time, delays in sowing cannot be seriously advised at the present time.

Chemical Control.

The history of chemical control measures against *Busseola* in South Africa has been reviewed by Jepson (1954), and details of a satisfactory method of control in South Africa, using 2.5 per cent. DDT dust, are given by Taylor (1952).

TABLE V.

The effect of dusting with 2.5 per cent. DDT dust against *Busseola* on maize in African-owned plots, Nzega, Western Province, 1954-55.

Plot no.	Total no. of plants at harvest		Area of sub-plot (sq. yd.)	Percentage of plant hills damaged by <i>Busseola</i> 34-40 days after germination		Calculated yield of shelled maize per acre (kg.)	
	Control	Treated		Control	Treated	Control	Treated
1	464	336	180	20.0	0.4	120.9	201.6
2	448	531	180	35.5	1.0	94.1	430.2
3	478	394	164	51.2	11.8	457.4	826.3
4	309	474	200	65.2	4.3	278.3	496.1
Mean	424.7	433.7	181	42.98	4.37	237.7	488.6

Difference between means required for significance ($P = 0.05$): 24.76, 376.47.
Dust applied at 10 lb. per acre 3 times, at weekly intervals, beginning about 21 days after germination.

Duerden (1953) reported the results of experiments against *Busseola* and *Chilo zonellus* at Kongwa, Tanganyika, in 1952. The treatments comprised a 3 per cent. DDT dust at 20–25 lb. per acre and sprays of 0.5 per cent. derris or 0.1 per cent. parathion at 20 gals. per acre. All three were applied once when the plants were three weeks old and gave limited protection only. It is clear from the biology of *Busseola* in Tanganyika that chemical protection is required for a period as long as that of the egg-laying period, approximately three weeks, beginning when the plants are about three weeks old and that, as the insecticides available at the present time have only brief persistence under tropical conditions, more than one application is required.

Field trials were carried out in 1955 on peasant holdings in the Nzega district of Western Province and in the Mbeya district of Southern Highlands Province, using 2.5 per cent. DDT dust applied three and four times, respectively, at weekly intervals.

In the Nzega district, 20 typical peasant holdings were selected in early January 1955, shortly after cultivation and sowing had been carried out. In all cases the crops consisted of mixed plantings of "Katumbili" maize and long-term "Wiru" sorghum, together with groundnuts. The plots, which were tieridged, were divided along the ridge into two approximately equal sub-plots, "treated" and "control". Three applications of a dust containing 2.5 per cent. DDT in diatomite were made, at weekly intervals, beginning as nearly as possible 21 days after germination of the maize. Ten pounds of the dust were applied per acre; both maize and sorghum plants were dusted.

Maize in 14 of the 20 plots was so badly affected by a combination of poor soil conditions and a drought in February that they were abandoned. It was observed that all of the six plots that withstood the drought had received variable amounts of kraal manure before sowing. Two of these plots were harvested by the owners without reference to the Department of Agriculture. On the four remaining plots, counts made 34–40 days after germination showed (Table V) that dusting significantly reduced the percentage of plant hills damaged by *Busseola*. The calculated average yield per acre of the treated plots (488.6 kg.) did not differ significantly from that of the control plots (237.7 kg.), but there was much variation between the plots. This variability may have been due partly to the fact that the plots were carrying crops of sorghum and groundnuts that competed with the maize, and partly to local differences in the rate of application of the kraal manure.

Little consideration has been given to the control of *Busseola* in the long-term "Wiru" sorghum of Western Province. The variety has an extremely long vegetative period, December to July, and is exposed to two generations of stalk-borer. By the time the second generation appears, the height of the sorghum is such that no simple method of control suited to the peasant farmer would prove adequate. The replacement of the long-term "Wiru" sorghum by the quick-maturing and shorter varieties now being introduced offers more hope of chemical control in this crop.

In the Mbeya district, Southern Highlands Province, the agricultural risks attached to maize growing are less than those in the Western Province and higher basic yields can be expected. Even here, however, maize in some of the experiments failed to mature owing to a combination of poor soil conditions and badly distributed rainfall.

Trials were carried out on typical African-owned plots, results being available from a total of 13. Each plot was divided into a treated and an untreated sub-plot, the areas of which varied from $\frac{1}{4}$ to $1\frac{1}{2}$ acres. In all cases the crop consisted of a pure stand of maize sown either on the flat or, more frequently, on the ridge. Plant-hill numbers averaged 4,500 per acre with 4–8 plants per hill. A dust containing 2.5 per cent. DDT in diatomite was applied by hand

duster into the funnel of each plant at the rate of 10 lb. per acre, four times, at weekly intervals, beginning as nearly as possible 18 days after germination of the maize. Sowings had been carried out as usual with the beginning of the rains in December. The first ten of the trials germinated in the period 10th to 31st December 1954, the last three between 11th and 26th January 1955. The data in Table VI show that, 70-75 days after germination of the maize, there

TABLE VI.

The effect of dusting with 2.5 per cent. DDT dust against *Busseola* on maize in African-owned plots, Mbeya, 1954-55.

Plot no.	Total plant hills in each sub-plot	Hills damaged 70-75 days after germination of maize (%)		Weight of maize cobs per 100 bearing plants (kg.)		Increase in yield (kg.)
		Treated	Control	Treated	Control	
1	1100	17.6	20.8	14.5	9.1	5.4
2	1025	1.8	53.3	23.6	10.9	12.7
3	1375	1.2	18.5	17.2	13.6	3.6
4	1500	0.3	13.8	12.7	9.1	3.6
5	1000 (T)* 1050 (C)†	3.3	45.5	38.5	8.2	30.3
6	950	7.3	39.2	29.9	10.0	19.9
7	670	11.6	33.2	34.5	14.5	20.0
8	1122	6.0	45.4	26.8	11.8	15.0
9	615	5.2	19.2	21.8	14.5	7.3
10	360	2.2	16.9	20.0	17.2	2.8
11	594	1.7	8.2	12.7	12.7	0.0
12	760	1.4	17.4	25.4	17.2	8.2
13	—	—	—	19.1	13.2	5.9
Mean		4.96	27.61	22.82	12.46	10.36 ± 2.37

Least significant difference in yield: 4.88.

Dust applied at 10 lb. per acre, 4 times, at weekly intervals, beginning about 18 days after germination.

* T = treated sub-plot; † C = control sub-plot.

was a mean of approximately 22.6 per cent. more damaged plant hills in the untreated than the treated plots. Yields were taken on cob-bearing plants, of which 100 were picked at random from each of the treated and untreated plots. Only the weight of the cob, not the weight of the shelled maize, was recorded. The increase resulting from the dusting was from 12.46 to 22.82 kg. of maize cob per 100 plants, an increase of 83.1 ± 19.0 per cent.

Discussion.

Whilst increased yields have been obtained as a result of controlling *Busseola* in maize it must be appreciated that there are factors other than this pest which affect the yield considerably. Rounce (1949) reported large increases in yield of maize, sorghum and *Pennisetum* in the Western Province from the application of various organic manures at the rate of 1-6 tons per acre. Tuckett (1955) concluded, from a time-of-sowing trial at Iringa in the Southern Highlands Province, that "Eckstein" maize sown on the earliest date, 23rd December, significantly outyielded all later sowings, 30th December to 10th February. A reasonably good yield from this variety of maize was obtained with as little as

7 or 8 inches of rainfall during its growing period, provided that this was evenly distributed.

The practice of manuring is as yet not very highly developed in peasant agriculture in Tanganyika and delays in sowing are occasioned by cultivation being in large measure dependent upon the hoe, so that in many cases only low basic yields can be expected, even when the rainfall is suitable. Under these circumstances, outlay on insecticides can hardly be justified. Where, however, conditions are good, with highly fertile or well-manured soils, early planting and well-distributed rainfall, high basic yields can be expected. The incidence of stalkborer damage is likely to be high in these early plantings, and under such conditions chemical control would probably be worthwhile.

Summary.

Busseola fusca (Fuller) is a serious pest of maize and sorghum in Tanganyika where it occurs mainly at altitudes of 4,000 ft. and over.

Laboratory work on the life-cycle and on the diapause of the larva is described. Diapause is terminated by contact with water.

Field work at Nzega, Western Province, indicates that the first sowings, made within two to three weeks of the beginning of the rains, are likely to be heavily attacked by larvae of the first generation. These are the progeny of a "rain-induced" flight of adults of *Busseola* derived from diapause larvae in the maize and sorghum left in the fields from the previous season, the larvae being induced to pupate when the stems are wetted by rain.

The number of generations in the main crop season, November–June/July, is two. A third generation occurs in sorghum tillers produced after June/July.

No alternate hosts of importance comparable with maize and sorghum have been found.

Cultural control by burning the crop residues left in the field after harvest, in order to destroy the diapause larvae, is not practicable in peasant agriculture as these residues are required for such purposes as palisading, building of contour banks and for grazing cattle and goats during the dry season.

Chemical control of the first generation of *Busseola* in young maize can be achieved by the application of a dust containing 2.5 per cent. DDT into the funnel of the plant. In 13 experiments on peasant-owned plots at Mbeya, in which a dust containing 2.5 per cent. DDT was applied four times, at weekly intervals, beginning about 18 days after germination, and at the rate of 10 lb. per acre, the average weight of maize cobs from 100 plants was increased from 12.46 to 22.82 kg., an increase of 83.1 ± 19.0 per cent. Factors other than *Busseola* which affect the yield are discussed and it is suggested that control measures would probably be worthwhile only where a combination of a suitably fertile soil, early planting and adequately distributed rainfall obtains.

Acknowledgements.

I wish to acknowledge the assistance of Mrs. C. A. Wyatt in breeding *Busseola* in the laboratory. My thanks are also due to Mr. C. C. Shapland, Provincial Agricultural Officer, Tabora, for considerable help with the experiments in his area and to Mr. R. C. Faun, Field Officer, who personally supervised all the experiments at Mbeya.

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QUANTITATIVE STUDIES ON TYROGLYPHID MITE POPULATIONS.

I.—THE DETERMINATION AND SIGNIFICANCE OF THE EGG DENSITY.

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The quantitative study of the physical ecology and essential requirements of Tyroglyphid mites appears to have received relatively little attention, although they are serious pests of stored flour and other cereal products. This may be due to the practical problems inevitably associated with routine population studies of small animals under controlled conditions, particularly when on a quantitative basis.

The necessity to separate the populations from the food material on which they subsist is one such problem, and, as Solomon (1945) has pointed out, this is a prerequisite to the accurate determination of population densities of flour mites. The published methods of separation, suitable for the treatment of flour, suffer from certain disadvantages which render them unsuitable for the present purpose, as will be indicated later.

In quantitative experiments in which the weight of the medium supporting the population is involved, especially those conducted at several humidity levels, variation of the moisture content is a complication. Further problems are connected with the sampling of cultures in which the population is unevenly distributed.

The present work is concerned with the solution of these problems and the design of a quantitative technique. This technique has been applied successfully to the study of Tyroglyphid populations at the egg stage and the results of experiments using *Thyreophagus entomophagus* (Lab.) are reported.

The Preparation of Infested Flour for Examination.

The existing methods of treating infested flour have been adequately reviewed by Solomon (1945) and therefore will be referred to only briefly. Berlese funnel techniques depending on the controlled application of heat to the infested flour and collection of the mites which migrate from the untenable conditions imposed on them, have been described by Chernuishev (1938) and Petrova (1940). The latter worker claimed the recovery of from 85 to 92 per cent. of the living mites present within 25 minutes. These techniques tend to be time-consuming and since only the mobile stages of the population are separated, they provide no direct information on oviposition.

Methods which depend on floating the mites (density about 1.0) from the flour (density about 1.4) using a liquid of intermediate density have also been described. Shchastny (1939) used a 1:1 brine/glycerol mixture for this purpose, after first treating the flour with ethanol; separation of the mites was hastened by centrifuging. Solomon (1945) employed dichlorethylene with which the flour was agitated in a long-necked flask; the mites were raised almost to the top of the neck by the addition of further fluid and removed on small pieces of filter paper. These methods are open to the objection that a proportion of the mites may remain entangled with the flour and no experimental work to test their efficiency has been reported. In the writer's experience, hatched eggs cannot be separated by these methods, which were therefore abandoned as inherently unsuitable for the present purpose.

The foregoing techniques have in common the object of separating the population from the flour. The alternative approach of separating the flour, or at least the greater part of it, from the population by chemical means was adopted by Smirnov (1938). Plain flour of commerce consists mainly of starch and protein, only about 5 to 10 per cent. of the dry weight being cellulosic, hence removal of the former effects a considerable reduction in the bulk. Smirnov treated the flour with antiformin and obtained the population in the centrifugate from the resulting mucilage. The process is somewhat time-consuming in that it requires from 12 to 24 hours for adequate digestion. For quantitative purposes, it has the further disadvantage that the centrifugate is slimy, due to the presence of incompletely gelatinised starch which hampers decantation.

Starch is rapidly hydrolysed by boiling dilute mineral acid which also dissolves part of the cereal protein, the remainder of which is soluble in cold, very dilute, alkali. Mites and their eggs are not visibly affected by this treatment, hence experiments along these lines were made and a satisfactory method developed which is described in detail below.

The Quantitative Basis.

If a method is to be quantitative, the weight or in some cases the volume of the sample of which the population has been counted, must be determined. The weighing of the sample is complicated by the inevitable change in moisture content which occurs during the process and the comparison of experiments at different humidity levels is invalidated unless moisture contents are also determined. Furthermore, when any considerable number of determinations is contemplated, weighing is too time-consuming and tedious to be practicable. These difficulties were overcome by the use of the lycopodium method of Wallis (1920). This method is now firmly established for the quantitative study of plant material but would appear to have been neglected in the entomological field. It depends on the great uniformity in size and weight of the spores of *Lycopodium clavatum*, of which 89,300–101,600 (mean 94,000) weigh one milligramme. Hence, if lycopodium spores be mixed in known proportion by weight with the flour used for cultures, it is then only necessary to count the numbers associated with the population in order to calculate the weight of flour originally concerned. By this method only one initial weighing is required and the results of experiments at different humidities can be directly related to one another. Furthermore, where large populations are concerned, an aliquot can be obtained with reasonable accuracy. Lycopodium spores are very resistant to chemical treatment and, therefore, even if they proved palatable to mites, which has not been observed to be the case, they would appear in the excreta unchanged. They are also non-hygroscopic, hence if one sample were used throughout an experiment, any inaccuracy due to variation of the moisture content or spore number per milligramme would be insignificant.

Selection of a Parameter.

The selection of a parameter suitable for ecological studies requires some consideration. Much of the published work in this field commonly expresses population density as the number of mites per unit weight of food material. Chemical treatment of infested flour or the use of one or other of the published flotation methods results in the death of the mites, thus permitting only a count of the total numbers present, irrespective of whether they were alive or dead before the treatment. This is unimportant if the difference between counts after a known time interval is determined, as it is the change in the population which is of interest. The counting of mites is complicated by the presence of cast skins which are not always easy to distinguish from intact mites at first.

sight. Disadvantages of this sort do not apply to the counting of eggs which, whether hatched or unhatched, are easy to recognise and distinguish at a glance from other structures present. The difference in the number of hatched eggs after a known time interval is indicative of the number of new individuals added to the population. When expressed in the form $\Delta N/\Delta t$, where ΔN is the number of new individuals added in time Δt , it becomes the natality rate per unit time as defined by Odum (1953). This is a parameter which may vary with the environmental conditions and also with the characteristics of the population. Lack of information on the latter need not invalidate its use as an index for comparing the effects of changing the environment, provided replicate populations are employed in the experiment. If at the same time the unhatched eggs are also counted, information is obtained on the rate of turnover of eggs. Consideration of both sets of data together may provide information on the ultimate ability of the population to survive. For example, a continuing decline in the number of unhatched eggs without a simultaneous increase in the combined totals must result in the extinction of the population. It therefore seemed advantageous to base subsequent work on counts of eggs both hatched and unhatched.

Preparation of the Culture Medium.

Cultures were reared on 100 per cent. extraction wheat flour from which the coarser particles were removed by shaking through a 60-mesh sieve. This was done to render the particle size more uniform and to facilitate subsequent copying of the medium. The sifted flour was mixed with 0.1 per cent. of its weight of lycopodium in a hand-mortar and then transferred to a screw-capped jar capable of holding about three times the quantity being dealt with. The jar was attached to a small geared-down electric motor so that it rotated about its longer axis at six revolutions per minute and was left running for about 36 hours. By this method, the flour rolled over and over upon itself continuously and thus was mixed with the lycopodium.

Preparation for Microscopical Examination.

The following method was developed after numerous trials with more complicated procedures. The number of transfers from one vessel to another has been reduced to a minimum to lessen the risk of any loss of material that might occur during transfer. A clearing treatment with chloral hydrate solution was included to prepare the residue for microscopic examination; this cleared residue may be preserved indefinitely after mixing with a suspending fluid. The procedure as described is suitable for the treatment of 0.5 to 2 grammes of flour, and in practice it has been found convenient to treat up to eight samples at one time.

Method.

The sample was boiled with 25 ml. of 5 per cent. hydrochloric acid in a 100-ml. conical flask for three to four minutes with occasional shaking, and then cooled. This hydrolysed the starch to soluble sugars and dissolved part of the cereal protein leaving the remainder as a flocculent suspension. A 50 per cent. w/w solution of potassium hydroxide was added, a few drops at a time, with shaking after each addition, until the liquid suddenly cleared, due to solution of the suspended protein. It was then transferred to a 50-ml. centrifuge tube and any residue in the flask was rinsed into this tube using about 20 ml. of water from a wash-bottle. The tube was centrifuged for several minutes and the clear supernatant liquid poured off as completely as possible. Two ml. of the clearing agent, a 5 in 2 w/w aqueous solution of chloral hydrate, was added and the tube placed in a boiling water-bath for about five minutes and oscillated until the residue became transparent. The contents were then transferred to the final

container, a 5-ml. specimen tube, using three quantities of about one ml. of 70 per cent. ethanol to complete the transfer. Ethanol was used because, unlike water, it did not precipitate matter which had dissolved in the clearing reagent. The specimen tube was stoppered, shaken to mix the contents and centrifuged for several minutes. Finally, the supernatant liquid was decanted and about 0.5 to 1.0 ml. of a suspending fluid added according to the weight of flour treated. The suspending fluid used was a 1 in 2 aqueous dilution of a polyvinyl mounting medium (Hall, 1951), which had the advantage of rendering the mounts semi-permanent. Other similar media would doubtless be equally suitable provided they remained clear on dilution with water.

Method of Counting.

The method of counting depends to some extent on individual preference and the type of microscope available. This should, however, be fitted with a calibrated mechanical stage to permit systematic examination of the whole area under the cover glass. The eye-piece was fitted with a disc ruled with parallel lines, the spacing of which was equivalent to 0.5 mm. on a stage micrometer observed through the 16 mm. objective. Slides were prepared by spreading one drop of the well-mixed suspension evenly over an area slightly less than $5/8$ in. square and applying a $5/8$ -in. square (16 mm.) cover glass. The spores were counted in eight scans 0.5 mm. wide spaced at 2 mm. intervals across the mount and the length of the cover glass measured with the aid of the calibrated stage. From these data the number of spores in the mount was calculated and hence the weight of the original culture represented, using the fact that 94,000 spores weigh 1 mg. The whole area of the mount was then scanned systematically and all the hatched and unhatched eggs counted. Unhatched eggs were distinguished by the ellipsoid shape and dense content. Occasional ones which were empty except for a clear yellow deposit at either end were included in the count, although probably infertile. Hatched eggs consisted of the empty but still intact egg membrane which was narrow ovate in shape and showed a longitudinal cleft with somewhat incurled edges.

The usual practice in quantitative microscopy is to count two slides for each suspension and take the arithmetic mean of the results, provided the difference between them is not more than 10 per cent. of the mean. Where the difference exceeds 10 per cent., two further slides are counted. To test this practice when using the above method of counting, duplicate slides were prepared from 20 cultures of which the total egg counts ranged from 20 to 1600/0.02 g. The resulting differences ranged from 0.54 to 19.4 per cent. (mean 8.5%) and there was no significant correlation with the total egg count. In seven cases the difference exceeded 10 per cent., indicating that about one-third of all slides may be expected to require counting again.

Population Distribution within Cultures and Sampling Technique.

Solomon (1946) has observed that mites which had been mixed thoroughly with flour and left undisturbed in a glass jar for 24 hours, tended to concentrate at the glass and the free surface of the flour. This took place both in darkness and on exposure to light. It seemed likely, therefore, that the egg distribution might also be uneven and, if this were so, it would influence the sampling technique in future experiments. The following experiment was designed to provide information on this point and also on the efficiency of the entire technique. All the flour used contained 0.1 per cent. w/w of lycopodium. A stock culture of *Tyroglyphus farinae* (Deg.) was mixed with flour by rotating in a screw-capped glass jar for 36 hours; then the surface was levelled and a small jar containing potassium hydroxide solution to maintain a relative humidity of 75 per cent. was

embedded in the centre of the flour. The culture was left undisturbed in a glass-fronted cupboard for three weeks. At the end of this period samples were taken at three points by plunging a 1-cm. diameter cork-borer through the flour and each core was divided into three approximately equal portions to represent the upper, middle and lower strata of the culture. The remaining culture was immediately killed by moistening with absolute ethanol which was allowed to evaporate and the dry material mixed by rotation for 36 hours. Nine samples were now taken, making 18 in all, and treated following the method described above. The numbers of hatched and unhatched eggs associated with 2,000 spores were counted, this being a convenient figure around the upper limit of the number of spores generally present on the microscope slide. It represented 2/94 or 0.021 g. of the culture. The results are recorded in Table I. They indicate that

TABLE I.

Egg distribution (numbers per 0.021 gramme) in a laboratory culture of
Tyroglyphus farinae on flour.

Hatched eggs								
Before mixing					After mixing			
Position	upper	Stratum middle	lower	Position means				
1	43.6	53.2	43.3	46.7	41.9	46.6	41.2	
2	33.9	25.9	42.9	34.2	38.5	39.5	36.4	
3	43.4	31.2	45.5	40.0	41.0	48.8	43.9	
Stratum means	40.3	36.8	43.9	40.3	42.0	Mean		

Unhatched eggs								
Before mixing					After mixing			
Position	upper	Stratum middle	lower	Position means				
1	10.2	11.8	5.7	9.2	6.1	6.4	3.3	
2	1.2	3.6	6.4	3.7	11.5	9.9	13.9	
3	3.9	4.2	0.6	2.9	8.5	8.1	7.3	
Stratum means	5.1	6.5	4.2	5.3	8.3	Mean		

the distribution of the hatched eggs in the undisturbed culture was uniform throughout the lower stratum, very uneven in the middle stratum and rather less uneven in the upper stratum. Unhatched eggs were unevenly distributed in all three strata with a tendency to accumulate in the middle stratum. For both hatched and unhatched eggs, the variance of the position means was greater than that of the stratum means. In this particular experiment, the ratio of unhatched eggs to hatched eggs was relatively low, hence the trends for the total counts (hatched and unhatched together) were not markedly different from those of the hatched eggs alone. The result of mixing and resampling the culture was a very marked reduction in the total variance although the means were in close agreement. Thus it was concluded that egg distribution in an undisturbed

culture was not uniform and that a single sample, taken at random even though it consisted of a complete vertical core, was unlikely to be representative.

In experiments conducted under controlled conditions, where samples have to be taken at regular time intervals, mixing of the culture prior to sampling is impracticable. This is due to the time required for adequate mixing, the difficulty of maintaining the conditions during this time and the unavoidable disturbance caused to the population. Hence it was decided that the best method of sampling would be to distribute the culture in a number of specimen tubes at the commencement. The whole contents of a tube could then be taken as a sample, with the minimum disturbance of the conditions of the experiment.

Error of the Technique.

The error of a single observation may be judged from the results for the mixed culture in Table I. For the hatched eggs, the standard deviation was 3.92 or 9.3 per cent. of the mean, and the maximum deviation of a single observation from the mean was 6.8 (*i.e.*, 48.8–42.0) or 16.2 per cent. of the mean. The corresponding data for the total egg counts were, standard deviation 3.39 or 6.75 per cent. of the mean, maximum deviation 6.6 (*i.e.*, 56.9–50.3) or 13.1 per cent. of the mean. The unhatched-egg counts were considered too small to provide a reliable estimate. The greatest difference between any two total egg counts was 12.4 (*i.e.*, 56.9–44.5) or about 25 per cent. of their arithmetic mean. Comparison with the data for duplicate counts recorded above, in which the greatest difference was 19.4 per cent., suggested that the error of the technique was associated mainly with the method of counting and that both the treatment of the flour and the method of mixing were adequate.

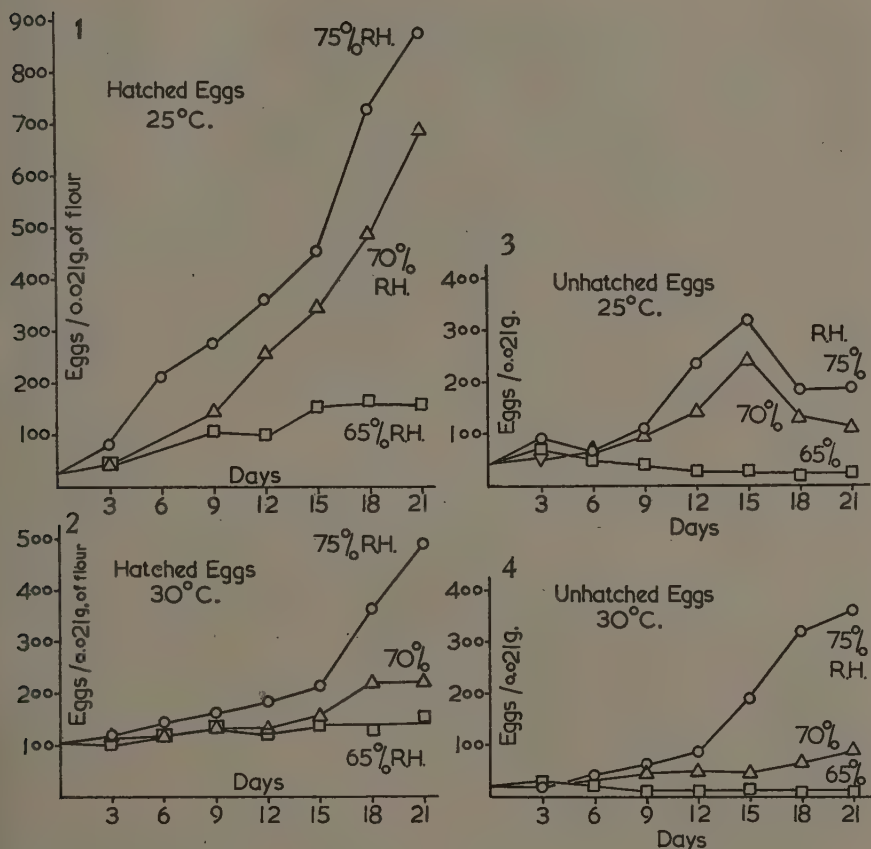
Application to Ecological Studies.

The above technique has been used over the past two years to study laboratory populations of *Tyroglyphus farinæ* under different combinations of temperature and humidity, and this work is still continuing. The following experiment, an earlier one using *Thyreophagus entomophagus*, is reported here to illustrate the application of the technique and the type of information it will provide. It was conducted at two levels of temperature and three of relative humidity, the latter being controlled by the use of potassium hydroxide solutions (Buxton & Mellanby, 1934).

The stock culture of *T. entomophagus* was maintained at 25°C. and 70 per cent. relative humidity. Prior to the experiment, a subculture was prepared by admixture with flour that had been conditioned at 70 per cent. relative humidity at the temperature of the experiment for the previous seven days. Although it was considered desirable that all experiments should be started simultaneously and with the same subculture, two subcultures were necessary because for practical reasons it was not possible to conduct experiments at more than one temperature concurrently. The subcultures were prepared from stock cultures, 8 to 10 weeks old, and diluted to approximately similar egg densities.

Mixing was done by rotation in a glass jar as described above, for eight hours. At intervals during this period the motor was stopped for a short time and the jar inspected for tracks against the glass. These always appeared within a few minutes, hence it was assumed that the rotation caused no injury to the mites. The subculture was distributed into 2 × 1-in. specimen tubes using a suitable spoon which, when levelled, delivered about one gramme. Seven tubes were accommodated around the circumference of a 4 × 3½-in. screw-capped jar and the central space occupied by a smaller jar containing the potassium hydroxide solution. Samples of the subculture were taken at the start of the experiment and one tube was removed from the jar every three days. If not prepared for

counting immediately the population was killed by addition of a few ml. of ethanol and prepared later. The spores and eggs were counted and the numbers associated with 2,000 spores calculated. Results are recorded graphically in figs. 1-4.



Figs. 1-4.—*Tyrophagus entomophagus*. The number of hatched and unhatched eggs/0.021 g. of flour (2,000 lycopodium spores) at 8-daily intervals. Results of an experiment, at 65, 70 and 75 per cent. relative humidity, conducted at 25°C. and repeated later at 30°C.

Discussion of Results.

At 30°C. and 65 per cent. relative humidity, the total numbers of hatched and unhatched eggs at each time interval was constant within the limits of experimental error, indicating that *T. entomophagus* did not oviposit under these conditions. The fall in the numbers of unhatched eggs and the coincident rise in the numbers of hatched eggs shown on the graph indicated that eggs present at the start of the experiment hatched slowly over a period of about nine days. At 25°C. however, the egg totals slowly increased for the first twelve days but the

graph of the unhatched eggs showed only an initial rise. This indicated that oviposition and hatching took place during the first half of the experiment and then ceased. Comparison of the slopes of the graphs of hatched eggs indicated that the natality rate at 65 per cent. relative humidity was greater at 25°C. than at 30°C.

At 30°C. and 70 per cent. relative humidity, oviposition continued throughout the duration of the experiment as shown by the increasing egg totals. The natality rate as indicated by the slope of hatched egg graph was low for the first half of the period but increased rapidly between the twelfth and eighteenth days after which hatching practically ceased. This cessation coincided with an increase in the number of unhatched eggs owing to the continuing oviposition. From this information it would appear that the lower limit of relative humidity for survival of the population was about 65 per cent. at 25°C. and 70 per cent. at 30°C.

The three remaining combinations of temperature and humidity displayed certain common trends. In each case hatching continued throughout the experiment, both the natality rate and the number of eggs hatched being greatest at 25°C. and 75 per cent. relative humidity and least at 30°C. and the same humidity. In each case also, oviposition tended to keep pace with hatching during the earlier part of the period, but lagged behind later, as shown by the early parallelism and later divergence of the graphs of hatched and unhatched eggs. This falling off in oviposition could be attributed to overcrowding in which case it would appear that at 25°C. a unit weight of flour could support a denser population at 75 than at 70 per cent. relative humidity.

Summary.

The quantitative study of Tyroglyphid populations involves their separation from the food material, for which existing methods are not entirely satisfactory. A new method is described which depends on chemical treatment to remove the bulk of the flour. It is made quantitative and independent of moisture-content variation by application of the lycopodium-spore method of Wallis. A technique for determining the numbers of hatched and unhatched eggs per unit weight of flour is described. It provides information on the rate of increase or decrease in the number of eggs (natality rate per unit time) and hence on the ability of the species to survive in a particular environment. The egg distribution in an undisturbed culture of *Tyroglyphus farinae* (Deg.) is found to be uneven. The technique is applied to the study of a population of *Thyreophagus entomophagus* (Lab.) under controlled conditions of temperature and humidity and the results discussed.

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THE UPTAKE OF DDT AND OTHER LIPOPHILIC PARTICLES BY BLOWFLIES WALKING OVER DEPOSITS.*

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In their experiments to establish the general principles of the contamination of insects exposed to particulate deposits Lewis & Hughes (1957) exposed blowflies to deposits of dye particles. Before their conclusions can be applied to insecticidal deposits, it is necessary to know the extent to which particles of a contact insecticide could be substituted for their lipophilic dye particles without altering the results. Measurements have therefore been made of the quantities of DDT picked up by blowflies from waxy, fibrous and oily substrates.

Results obtained by Lewis & Hughes (1957) suggest that the presence of oil hinders the uptake of particles from oily crystalline deposits of an insecticide, although these deposits are known to be more toxic than dry deposits (Barlow & Hadaway, 1952b). The uptake of DDT from oily substrates has, therefore, been studied further by the use of different quantities of oil and of different oils.

Work by Barlow & Hadaway (1952a), using substrates of glass, mud, plaster and wallboard, showed that the uptake of DDT may be reduced by the presence of dried wetting agent and that this reduction depends on the absorptive properties of the substrate. Because of the practical importance of this discovery, further experiments have been performed, using two methods of application of a different wetting agent to paper and wax substrates.

Materials and Techniques.

Deposits of particles of purified p,p'-DDT of mass mean dimensions 25 microns were obtained by the air sedimentation method used by Lewis & Hughes (1957). For the experiments with different oils and for some of those with the wetting agent, BDH oil-soluble violet dye particles of mass median diameter 20 microns were used, the deposits of these particles being obtained by the same method.

Filter papers or glazed cards were used as fibrous substrates and glass plates coated with carnauba wax as waxy substrates. Oily deposits were obtained by application of oil to fibrous substrates prior to dusting the surfaces with particles. Deposits on substrates contaminated with dried wetting agent were prepared in one of two ways: either the substrate was dusted with particles immediately after an aqueous solution of wetting agent had been applied to it and then allowed to dry, or the substrate was allowed to dry between the application of the wetting agent solution and the application of the particles.

Batches of five flies of the species *Phormia* (*Protophormia*) *terraenovae* R.-D. were exposed to dusted surfaces for short intervals of time in an exposure apparatus used by Lewis & Hughes (1957). The experiments were performed under a bright light in a constant temperature room at 25°C., under which conditions the flies walked throughout the period of exposure. The exposed flies were washed in a solvent and the quantity of material which they had picked up was measured by analysis of the washings. The quantity of p,p'-DDT was determined by Barlow & Hadaway's modification (1952a) of the colorimetric method of Schechter & others (1945). The oil-soluble violet dye was determined colorimetrically.

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(2490)

The Uptake of Dry Particles from Fibrous and Waxy Surfaces.

Flies were exposed to DDT deposits on surfaces of filter paper and of carnauba wax for different intervals of time between $\frac{1}{2}$ and 4 minutes. Since there was a variation in the density of the deposits to which different batches of flies were exposed, the uptake of DDT could not be compared without an adjustment being made to allow for this variation. Lewis & Hughes (1957) showed that the uptake of lipophilic dyestuff from surfaces of both filter paper and carnauba wax is directly proportional to the density of the deposit. The same relationship has been found to exist when deposits of DDT are used. Therefore, the results for the weight of DDT picked up during each exposure were adjusted for the variations in the density of the deposits on the basis of this linear relationship. The rate of uptake of DDT from each type of surface was shown to fall off during the first minute but then to remain constant.

These results for the uptake of DDT particles are compared, in fig. 1, with those obtained by Lewis & Hughes (1957) for lipophilic dye particles of similar size, the necessary adjustment being made for the different deposit density on

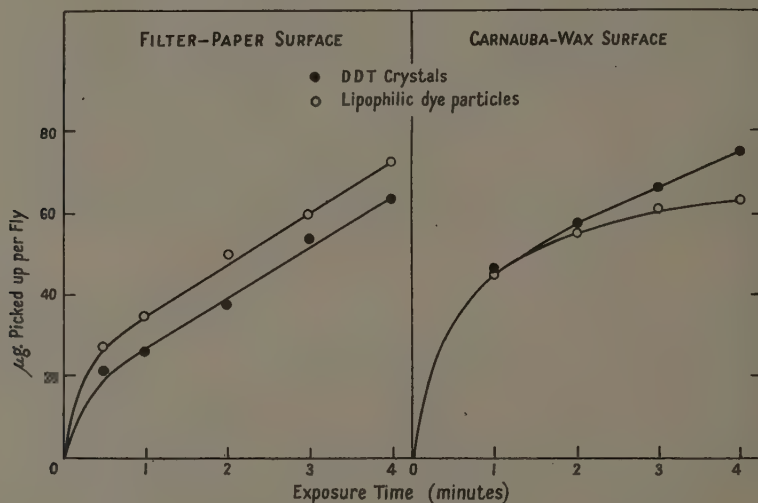


Fig. 1.—The variation of the uptake of DDT particles and lipophilic dye particles by blowflies with the length of exposure to deposits of $25\mu\text{g./cm.}^2$ on surfaces of filter paper and carnauba wax.

which Lewis & Hughes base their results. Since DDT particles differ in shape, density and oil-solubility from lipophilic dye particles, an exact correlation between the quantities picked up from deposits of each type of particle would be unlikely. However, the similarity in the uptake of the particles in these experiments shows that the adhesive behaviour of the two types of dry particle is similar, so that conclusions drawn from experiments with deposits of dry lipophilic dyestuff may be extended to deposits of dry DDT.

The Uptake of Oily Particles.

Flies were exposed to deposits of DDT on filter paper oiled with the white oil, Shell Risella 17 (previously known as P 31). The particles were wetted by

the oil but no observable solution occurred during the period of the experiment. The uptake of the DDT particles was seriously hindered by the presence of oil, since in two minutes a fly was found to pick up an average of $0.4 \mu\text{g.}$ of oily DDT (the average of 80 flies), which is only 1 per cent. of the quantity of dry DDT picked up in the same time.

The availability of DDT particles is far more affected by the presence of oil than is that of the dye particles, the rate of uptake being reduced by a factor of ten in the case of the dyestuff (Lewis & Hughes, 1957).

This difference might be thought to result from DDT being more lipophilic than the dyestuff (at 25°C. the solubility of DDT in Risella 17 is 20 g./l. and that of dyestuff 0.02 g./l.) but later experiments, using different oils, showed that differences in solubility are unlikely to have any effect on the uptake of particles. The shape of the particles may have a more marked effect on the uptake from oily deposits: needle-shaped DDT particles, when they lie horizontally, have a greater area of contact with the oil than isometric dye particles.

Experiments on the effect of different quantities of oil on the uptake of DDT from an oiled substrate were performed with deposits on a glazed card. The amount of oil applied to the substrate was found by weighing the cards before and after oiling. The smallest quantity of oil used was sufficient to penetrate the whole thickness of the card but the largest quantity was insufficient to saturate the card, judging from the absence of free oil on the surface.

Preliminary experiments showed that the length of time that the particles were in contact with the oil was important, the uptake of particles decreasing as the time interval after applying the dust increased, *e.g.*, $45.2 \mu\text{g./fly}$ were picked up after an interval of one hour compared with $20.8 \mu\text{g./fly}$, under similar conditions, after three hours. Since solution of DDT in the oil was negligible in this period of time, the decrease in uptake was attributed to creep of the oil round the particles, increasing their adherence to the substrate. As in experiments with dry particles (Gratwick, The Contamination of Insects exposed to

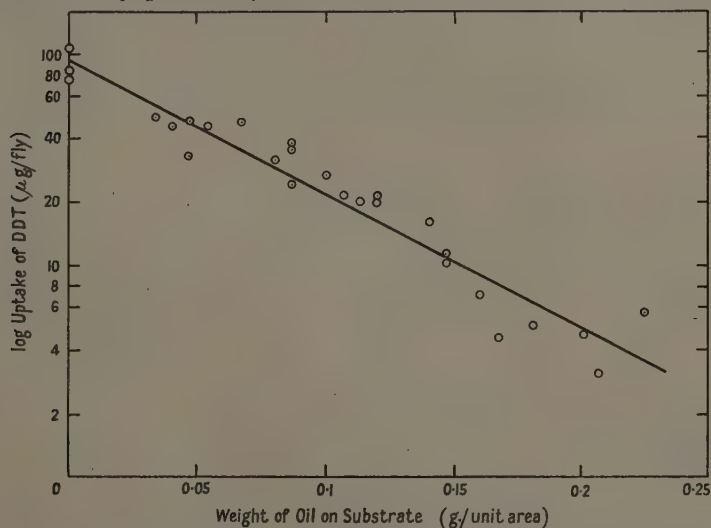


Fig. 2.—The variation of the uptake of DDT particles by blowflies, in one minute exposures, with the quantity of oil applied to a card substrate. The density of the DDT deposit is $25 \mu\text{g./cm.}^2$ The best fit regression line is drawn through the points.

Deposits of Lipophilic Particles. Unpublished Report to Colonial Pesticides Research Committee, 1956), there was a decrease in the availability of the deposit with successive exposures, even when only small quantities of DDT were picked up.

In the final experiments, deposits on a series of cards, dry and treated with different amounts of oil, were exposed to flies for one minute, after allowing one hour to elapse between dusting and exposure. Each deposit was used once only. Results (fig. 2) show that the logarithm of the weight of DDT picked up is inversely proportional to the weight of oil present.

Experiments using different oils were performed to determine whether the viscosity of the oil or the solubility of the particles in the oil are important factors in the uptake of lipophilic particles from oily deposits.

There was only a small difference, significant at the 5 per cent. level of probability, between the uptake of particles from filter paper oiled with Risella 17 and that from filter paper oiled with an oil (Risella 33) of widely different viscosity (Table I).

TABLE I.

Uptake of particles from filter papers treated with oils of widely different viscosity.

Oil applied to substrate	Viscosity (Redwood I secs. at 70°F.)	Average wt. ($\mu\text{g./fly}$) of dye- stuff picked up in 2 min. from deposit of density 25 $\mu\text{g./cm.}^2$
Risella 17	125	4.6
Risella 33	800	3.7

It was concluded that the viscosity of the oil has no more than a slight effect on the uptake of particles from oily deposits.

In an experiment using two oils of similar viscosity, the different solubilities of the dye particles in the oils had no significant effect, at the 5 per cent. level of probability, on the uptake of the particles from oiled substrates (Table II).

TABLE II.

Uptake of particles from filter papers treated with oils having widely different solvent action on the particles.

Oil applied to substrate	Solubility of dyestuff at 25°C. (g./l.)	Average wt. ($\mu\text{g./fly}$) of dye- stuff picked up in 2 min. from deposit of density 25 $\mu\text{g./cm.}^2$
Liquid paraffin	0.018	2.9
Olive and castor oil (2 : 1 mixture)	18.4	2.4

The Uptake of Particles associated with a dried Wetting Agent.

The uptake of particles from substrates to which a solution of a wetting agent had been applied and allowed to dry was compared with that from substrates to which no wetting agent had been applied. The wetting agent selected for these

experiments was Stergene, a commercial, non-ionic substance, prepared by the condensation of an alkyl phenol with ethylene oxide. Two sets of experiments were carried out, the deposits being prepared by a different method in each.

Particles applied to a wet substrate.

In the first experiments, substrates of filter paper and of carnauba wax were treated with wetting agent solution and dried after the application of the particles. When deposits were prepared by this method the uptake of lipophilic dye particles from filter paper was reduced by the dried Stergene by about 30 per cent. and the uptakes from substrates to which two different concentrations of Stergene solution had been applied were similar (Table III).

TABLE III.

Uptake of particles ($\mu\text{g./fly}$) from filter paper treated with wetting agent, dusted and then dried.

No Stergene residue	0.5 per cent. Stergene soln. applied	10 per cent. Stergene soln. applied
44.5	30.8	29.3

(Each figure is the average uptake for 30-40 flies exposed for 2 min. to deposits of density $25 \mu\text{g./cm.}^2$)

The reduction in the uptake of DDT was found to be of the same magnitude as that for the dyestuff.

The application of particles to a waxy substrate when the latter is wet renders the final deposit rather uneven, so that experimental replicates using deposits on wax prepared by this method were variable. The weight of DDT picked up from these deposits was approximately one ninth of that picked up in the absence of a residue of Stergene (a reduction of about 89%). Thus the uptake of particles from the non-absorbent, waxy substrate is far more affected by the addition of a wetting agent to it than is the uptake from the absorbent filter paper (*cf.* Barlow & Hadaway, 1952a).

Particles applied to a dried substrate.

The second set of experiments was performed using deposits which had been prepared without the wetting of the particles by the Stergene solution, *i.e.*, where the latter had been allowed to dry before application of the particles. Dried Stergene did not reduce the uptake of lipophilic dye particles from deposits prepared in this way on filter paper. The weights picked up from papers with

TABLE IV.

Uptake of particles ($\mu\text{g./fly}$) from wax treated with wetting agent, dried and then dusted.

No Stergene residue	0.1 per cent. Stergene soln. applied	0.5 per cent. Stergene soln. applied
79.2	37.7	40.0

(Each figure is the average uptake for 40 flies exposed for 2 min. to deposits of density $25 \mu\text{g./cm.}^2$)

a Stergene residue and from those without were 36.6 and 35.9 $\mu\text{g./fly}$, respectively. However, when the deposits were on carnauba wax the uptake of dye particles was reduced by 50 per cent. Again there was no appreciable difference in the uptake from substrates to which Stergene solution of two different concentrations had been applied (Table IV).

To sum up, a dry residue of Stergene considerably increases the adhesion of lipophilic particles to a non-absorbent, waxy substrate even when the particles have not been wetted by the Stergene solution but this increase in adhesion is not so great as when the particles have been wetted, the condition existing in the field when aqueous suspensions are used. An increase in adhesion to filter paper occurs only when the particles are applied to the wet substrate.

Discussion.

The availability of the particles in a deposit is determined by the adhesion of the particles to the substrate and to the insect cuticle, and the extent to which insects walking over the substrate come into contact with the particles.

Variation in the average adhesion of particles to substrates of different types is shown by a comparison of the uptakes of dry DDT, in one minute, from substrates of filter paper (fig. 1), of wax (fig. 1) and of glazed card (fig. 2). The reason for the greater uptake from the card compared with the waxy substrate is explained by differences in the affinity of the particles for the two substrates, lipophilic particles possessing a greater affinity for substrates of a lipid nature (Lewis & Hughes, 1957) and therefore being more readily picked up from a non-lipoid than a lipid substrate. The uptake from filter paper is less than from either of the other substrates because some of the particles lie in the interfibrillar spaces out of reach of an insect walking on the surface. Therefore, the difference in the physical structure of the filter paper and the hard waxy surface conceals the smaller affinity of the particles for the non-lipoid surface compared with the lipid one.

A difference in the average adhesion of particles to a substrate occurs when the substrate is modified by the presence of oil or a residue of wetting agent. Increased adhesion of DDT to a fibrous substrate in the presence of oil is probably caused by an increase in the area of contact of the particles with the substrate, as well as by lipophilic particles having a greater affinity for an oily than a dry substrate. Experiments have shown that this increase in adhesion depends on the amount of oil applied to the substrate. It is suggested that, as increasing amounts of oil are added to a fibrous substrate, the oil is absorbed into the fibres by capillarity and when these are saturated, it begins to fill the spaces between them. When these interfibrillar spaces are filled, the whole substrate is completely saturated. Since the substrate was not completely saturated by any of the amounts of oil used in these experiments, it seems probable that the difference in the cards to which different quantities of oil had been applied was in the extent to which the intrafibrillar or interfibrillar spaces were filled with oil. The flow of oil into the spaces of the substrate is probably a fairly slow process and may account for the greater adhesion of particles to the substrate when they have been lying on the oiled surfaces for a longer period.

Oily crystalline deposits of DDT are more toxic than dry crystalline deposits obtained by evaporation of a volatile solvent (Barlow & Hadaway, 1952*b*). This difference in toxicity, which cannot be due to a greater availability of the particles of oily deposits, is probably due to the increased rate of penetration through the insect cuticle of DDT in the presence of oil.

Lipophilic particles were shown to adhere more strongly to a non-absorbent substrate when wetting agent solution was allowed to dry on the surface before the application of the particles than to a non-absorbent substrate not so contaminated. This increase in adhesion may be due to a more complete contact

between each particle and the substrate or to the particles having a greater affinity for a surface contaminated with a residue of wetting agent than for an uncontaminated surface. On an absorbent substrate, no such increase in adhesion occurred when the particles were applied to the dried substrate, because the Stergene was carried into the substrate before the application of the particles. Contact between the particles and the wetting agent could not, therefore, occur. An increase in the adhesion of lipophilic particles to an absorbent substrate and a further increase in the adhesion of particles to a non-absorbent substrate occurred when the particles were applied whilst the wetting agent was still present as a solution. In this case the factor responsible for the decrease in the availability of the particles may be deposition of wetting agent on the particles, so that they do not adhere to the insect cuticle so well, or, more probably, to the accumulation of wetting agent around those surfaces of the particles which are in contact with the substrate, causing them to adhere more firmly to it.

Summary.

Estimations have been made of the weight of material accumulated by adults of *Phormia (Protophormia) terraenovae* R.-D. from deposits of DDT and other lipophilic particles on different types of substrate on which the flies were exposed.

Uptake of dry DDT particles is shown to be similar to that of dry lipophilic dye particles of similar size so that conclusions on the contamination of insects by dry lipophilic dyestuff may be extended to contamination by DDT dust.

The presence of oil greatly reduces the uptake of DDT particles from a fibrous substrate, the uptake decreasing logarithmically with increase in the amount of oil applied to the substrate. The viscosity of the oil has no more than a very small effect on the uptake of lipophilic particles from oily deposits and the solubility of the particles in the oil has no significant effect.

A reduction in the uptake of dry lipophilic particles from filter paper and from a hard, waxy substrate occurs when a wetting agent solution is applied immediately before the particles. When the particles are applied after the drying of the wetting agent on the substrate, a reduction in uptake also occurs on the non-absorbent, waxy surface, but is not so large as when the particles are applied before drying of the wetting agent. No reduction in uptake occurs on the absorbent substrate under these conditions.

On the basis of the experimental results the variations in the adhesion of lipophilic particles to substrates are discussed.

While differences in the affinity of particles for substrates of different types cause variations in adhesion, the physical structure of the surface may be more important in determining the amount picked up from it. The presence of oil or wetting agent on the surface increases adhesion, probably by increasing both the affinity of the lipophilic particles for the substrate and the area of contact between the particles and the substrate.

Acknowledgements.

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THE CONTAMINATION OF INSECTS OF DIFFERENT SPECIES EXPOSED TO DUST DEPOSITS.*

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Previous workers who have studied the accumulation of particles by insects from surface deposits have not compared the quantities picked up by insects of different species under similar conditions, and the only study of the distribution of particles picked up by insect tarsi is that made by Lewis & Hughes (1957) involving one species of blowfly, *Phormia* (*Protophormia*) *terraenovae* R.-D. A comparative study of the contamination of several species exposed to similar deposits of lipophilic dyestuff was therefore undertaken. The effect of redistribution and loss of particles on the proportions of dust present on various regions of the insect was also studied for one species (*Vespula vulgaris* (L.)), thus extending the work by Lewis & Hughes (1957) on the cleaning activity of blowflies.

Materials and Techniques.

The insects.

Insects were selected for variety of size and tarsal structure and for ability to walk over a dusted surface.

Wasps (workers of *Vespula vulgaris*, Hymenoptera, VESPIDAE) and ground beetles (*Feronia madida* (F.), Coleoptera, CARABIDAE) were chosen as an example of two insects which exhibit a marked contrast in the degree of hairiness of the

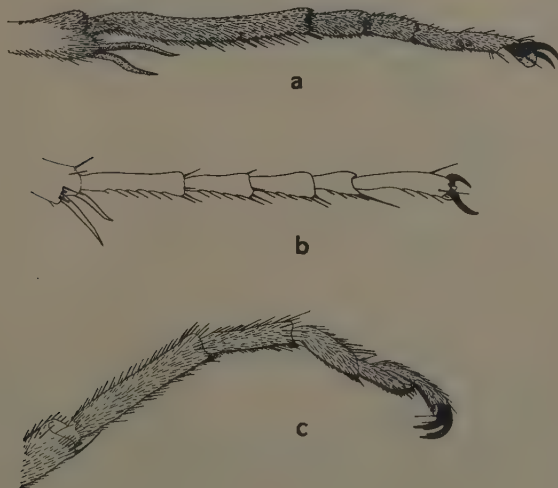


Fig. 1.—Diagrams of the lateral view of the hind tarsi of
(a) a worker of *Vespula vulgaris* ($\times 16$), (b) *Feronia*
madida ($\times 16$) and (c) *Rhagonycha fulva* ($\times 34$).

* This work forms part of a thesis approved by the University of London for the M.Sc. degree.

tarsi. The tarsi of wasps have a dense covering of setae, particularly on the ventral side, whereas the tarsi of *Feronia* possess a smooth, bare cuticle except for two rows of strong spines on the ventral side and a few around the distal edge of each segment (fig. 1). Soldier beetles (*Rhagonycha fulva* (Scop.), Coleoptera, CANTHARIDAE) were selected for comparison with *Vespula* and *Feronia* because they are considerably smaller and although, like *Vespula*, the tarsi have a dense covering of setae (see fig. 1) they are structurally peculiar in that the fourth segments are bilobed.

The above three species possess five tarsal segments. Two species with only three tarsal segments were studied for comparison. These were cotton-stainers (*Dysdercus fasciatus* Sign., Hemiptera, PYRRHOCORIDAE) and plant bugs (*Notostira erratica* (L.), Hemiptera, MIRIDAE) which exhibit a contrast in size while the degree of hairiness of the tarsi is similar (fig. 2). *Notostira* differs from the other insects studied in that the middle legs are shorter than the forelegs. Since the difference in weight between the two sexes of *Dysdercus* is more marked than in most species, these insects were also used to show the effect of differences in the weight of the insect in the absence of differences in the structure and size of the tarsi.

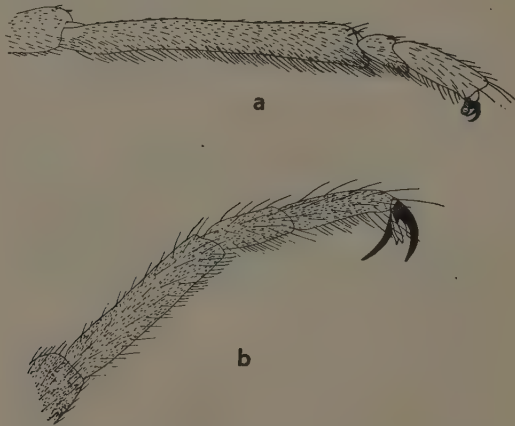


Fig. 2.—Diagrams of the lateral view of the hind tarsi of (a) *Dysdercus fasciatus* ($\times 30$) and (b) *Notostira erratica* ($\times 44$).

The deposits.

Strips of glazed card were dusted with dry particles of BDH oil-soluble violet dye by the air sedimentation method used by Lewis & Hughes (1957). The mass mean diameter of the particles was 20 microns. These dye particles were used instead of particles of contact insecticide because dyestuff can be determined more rapidly than insecticide, and previous experiments had shown that their adhesive properties are similar to those of dry DDT particles (Gratwick, 1957).

The exposure of the insects.

The insects were exposed to the deposits in perspex tunnels of triangular cross-section. A tunnel with base 12×2.8 cm. was used for most of the insects but the Mirids and a few of the wasps were exposed in a smaller one of base

6 × 2.2 cm. After a dusted strip of card had been placed on the floor of the tunnel, an insect was introduced at one end, allowed to walk the length of the dusted surface and removed at the far end. For experiments in which the quantity of dyestuff accumulated on the whole or part of the contaminated insect was to be determined, the part concerned was washed in a known volume of acetone and the weight of dyestuff determined indirectly by a colorimetric method. When the uptake per step was to be determined, the number of steps (throughout this work to be understood as the number of times that each tarsus of the insect is placed on the surface) taken to complete the distance was counted. For a study of the distribution of dyestuff on the tarsi, the particles on representative parts of all the tarsal segments of the contaminated insect were counted under a microscope.

A comparative Study of the Quantities of Dust picked up.

Total uptake.

The dust accumulated by insects walking short distances over dusted surfaces was expressed as the average weight picked up at each step. Variations in the speed at which an insect walks were found to have no effect on the average uptake per step, although uptake per unit distance varies because of differences in the length of stride of an insect at different speeds. Within the range of the experiments, variations in the number of steps taken were found to be unimportant.

Results for the average uptake per step for each of the five species indicate that there is a slight variation from one species to another (Table I).

TABLE I.

The uptake of lipophilic dye particles by insects of different species.

<i>Insect</i>	Average uptake per step (μg.)	Average weight of insects (g.)	Average uptake (μg.) per step per g. body weight
<i>Feronia</i>	1.7	0.147	11.6
<i>Vespula</i> (worker) ..	1.2	0.080-0.105	11.4-15.0
<i>Dysdercus</i> (female) ..	1.5	0.088	17.0
<i>Dysdercus</i> (male) ..	1.2	0.047	25.5
<i>Rhagonycha</i>	1.1	0.020	55.0
<i>Notostira</i>	1.0	0.006	167

The uptakes per step are the average for 11-24 insects which have walked 10-20 steps over deposits of density 80 μg./cm.² The weights of the insects are averages for 6-12 live insects.

The least significant interval in the means of the quantities picked up per step by each species was found to be 0.4 μg. (at the 5% level of probability) so that any two insects whose mean uptakes differ by this amount pick up significantly different quantities. On this basis the ground beetles (*Feronia*), despite lack of hairs on the tarsi, pick up significantly more than all the others except female cotton-stainers (*Dysdercus*) whilst female cotton-stainers pick up significantly more than soldier beetles (*Rhagonycha*) or the Mirids (*Notostira*). This result shows that differences in uptake between insects of varying size and structure, particularly in the structure of the tarsi and in the degree to which

they are clothed with setae, are not large and that existing differences appear to be due to differences in size, large size favouring uptake. Although females of *Dysdercus* are almost twice the weight of the males, while the tarsi are of similar size, the amounts picked up by the two sexes are not significantly different. Thus a difference in weight alone must be very large to have a significant effect. Because of this small variation in uptake with the weight of the insect there is a very great variation in the uptake per unit body weight for insects which differ in size. In the range of insects studied, an increase in size was accompanied by a decrease in the number of particles picked up per unit body weight (Table I) indicating that a small insect may collect a toxic dose of insecticide during fewer steps on a deposit than a large one.

Uptake by fore-, middle and hind legs.

The relative importance of the fore-, middle and hind legs was found for each species by determining the proportion of dyestuff picked up by each pair (Table II).

TABLE II.

The proportions of lipophilic dye particles picked up by the fore-, middle and hind pairs of legs.

Insect	Uptake expressed as a percentage of the total on the legs		
	Fore	Middle	Hind
<i>Vespula</i> (worker)	24	25	51
<i>Feronia</i> (male and female)	27	21	52
<i>Dysdercus</i> (male)	17	26	57
<i>Dysdercus</i> (female)	16	29	55
<i>Rhagonycha</i> (male)	20	25	55
<i>Rhagonycha</i> (female)	15	26	59
<i>Notostira</i> (male and female)	25	25	50

Each set of figures is the average for at least five insects.

The main conclusions are (1) that there is a marked similarity in the ratio between the quantities of dye picked up by the fore-, middle and hind legs, respectively, in the different species; (2) that the total weight of the insect does not affect these proportions, although differences between the sexes of *Rhagonycha* may be due to the relatively larger and heavier abdomen of the female and (3) that in all cases the hind legs pick up an amount equal to or greater than the sum of the amounts picked up by the other two pairs.

The Distribution of Particles picked up by the Tarsi.

An estimate was made of the relative number of particles accumulated by different segments of the tarsi and pretarsi of all the legs of eight individuals of each insect species. Each tarsus was viewed from one side and only particles that were visible without moving it were counted. One of each pair of tarsi was viewed from the antero-lateral side and the other from the postero-lateral side, so that any bias due to particles being picked up asymmetrically about the dorso-ventral axis was eliminated.

Percentage uptake.

The distribution of particles on the tarsi of each species is shown by a comparison of the average percentage uptakes of the different segments. The percentage uptake on a particular tarsal segment of an individual insect is here taken to be the sum of the number of particles on corresponding segments of a pair of tarsi expressed as the percentage of the total number counted on all the tarsi of that insect. Histograms showing the average percentage uptakes for the tarsal segments and the pretarsi of the five species studied in this work are given in fig. 3. Histograms for *P. terracnovae*, plotted from figures obtained by Lewis & Hughes (1957), are included for comparison.

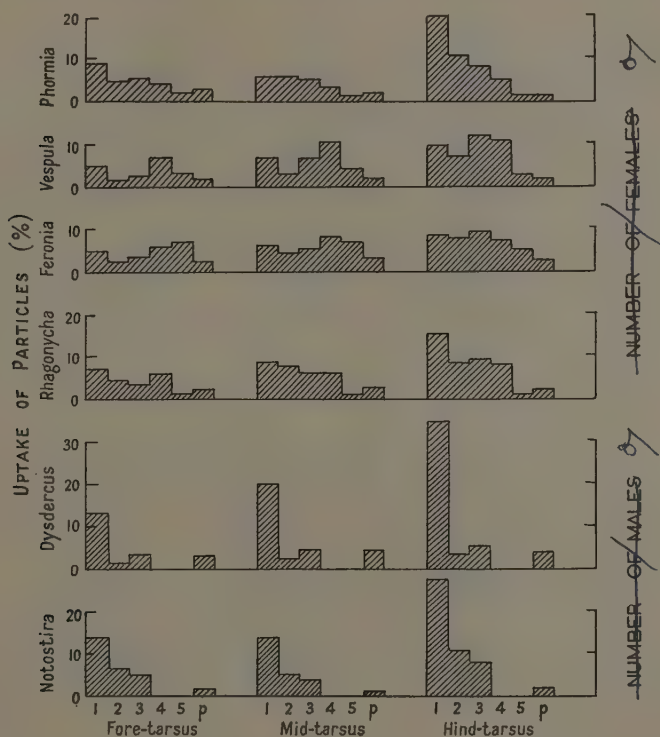


Fig. 3.—The average percentage uptakes of the tarsal segments and the pretarsi.

In all the species the hind tarsi are the most important accumulators of particles, which was to be expected from the knowledge that half of the total quantity picked up by the legs is found on the hind pair. The first segment of each of the hind tarsi of *Phormia*, *Rhagonycha*, *Dysdercus* and *Notostira* picks up considerably more than any other tarsal segment of the same insect but the uptake of *Vespula* and *Feronia* is more evenly distributed so that no segment picks up a much higher proportion than any other.

The other outstanding feature of uptake is the small amount picked up by the pretarsi. The percentage uptakes of the pretarsi are as follows: *Notostira*, 5.6,

Vespula, 5.7, *Phormia*, 6.0, *Rhagonycha*, 6.5, *Feronia*, 8.1, *Dysdercus*, 11.0. *Rhagonycha* and *Feronia* lack any form of pretarsal lobes ventral to the paired claws while the other species possess lobes of different types. Thus, *Vespula* has a single, median, pad-like arolium on each pretarsus, *Dysdercus* has paired, knob-like arolia, *Notostira* has paired, flattened, membranous structures which are known as arolia but it is uncertain whether they are strictly homologous with the arolia of other orders (Holway, 1935), and *Phormia* has paired, pad-like pulvilli. The four species whose pretarsi pick up similar percentages exhibit marked differences in the structure of the pretarsi which might have been expected to cause large variations in the percentage uptake. Thus pretarsi which possess pulvilli or arolia do not pick up a higher percentage of particles than those which lack them.

Collecting efficiency.

The length of each tarsal segment was measured so that the percentage uptake per unit length could be calculated. The setae projecting beyond the distal end of the segments were not included in these measurements, nor were the claws included in the pretarsal measurements, which were confined to the pretarsal lobes. The uptake per mm., as a percentage of the total uptake for all tarsi, is called the collecting efficiency. Histograms for the average collecting efficiencies of the tarsal segments and pretarsal lobes (not the whole pretarsus as in fig. 3) of the five species studied are given in fig. 4. Histograms for *Phormia terraenovae* plotted from figures quoted by Lewis (1954b), are included for comparison.

Differences in the collecting efficiency of tarsal segments of an insect are due either to structural differences or to differences in the part played by the segments in walking. Where the structure is similar, differences in collecting efficiency are assumed to be due to differences in thrust (*cf.*, Lewis & Hughes, 1957). The relative collecting efficiencies of the tarsal segments of each species are interpreted on this basis.

The hind tarsi as a whole are generally the most efficient while the fore-tarsi are the least. This phenomenon may be explained by differences in the thrust exerted by the different tarsi. *Phormia* differs from the other five species in that the middle tarsi are the least efficient, which Lewis (1954b) attributes to the relatively small number of ventral setae on the first two segments.

Five-segmented tarsi.—The middle segments of five-segmented tarsi are generally more efficient than the proximal or distal segments because the greatest thrust is exerted by the middle part of these tarsi. The high efficiency of the fourth segments of all the tarsi and of the third of the hind tarsi in *Vespula*, *Rhagonycha* and *Feronia* probably reflects the importance of these segments in walking. In *Phormia* the proximal segments appear to be more important than in the other species.

In both *Vespula* and *Feronia* there is an increase in the efficiency of the second and third segments and a decrease in the efficiency of the fifth segment from the fore- to the middle to the hind tarsi, which cannot be explained by differences in the density of the ventral setae but could be explained by differences in the centre of thrust (the point about which pressure on the surface is equally distributed). In both these species the centre of thrust has been found to be situated three-quarters of the way along each tarsus. Measurements show that there is a disproportionate increase in the length of the tarsal segments towards the posterior end of the insect, so that the centre of thrust of each tarsus is in a different position relative to the tarsal joints. The apparent shift in the centre of thrust is towards the proximal end of the tarsus as the tarsal length increases and, therefore, can account for the differences in the collecting efficiency of the second, third and fifth segments of the different tarsi of *Vespula* and *Feronia*.

In *Rhagonycha* the bilobed fourth tarsal segments are always the places of maximum thrust. However, there is a progressive increase in the efficiency of the first three tarsal segments from the anterior to the posterior end of the insect which, in the absence of structural differences, is attributed to a corresponding increase in the thrust applied to the substrate causing an increase in the over-all area of contact with the substrate. The fifth tarsal segments of this

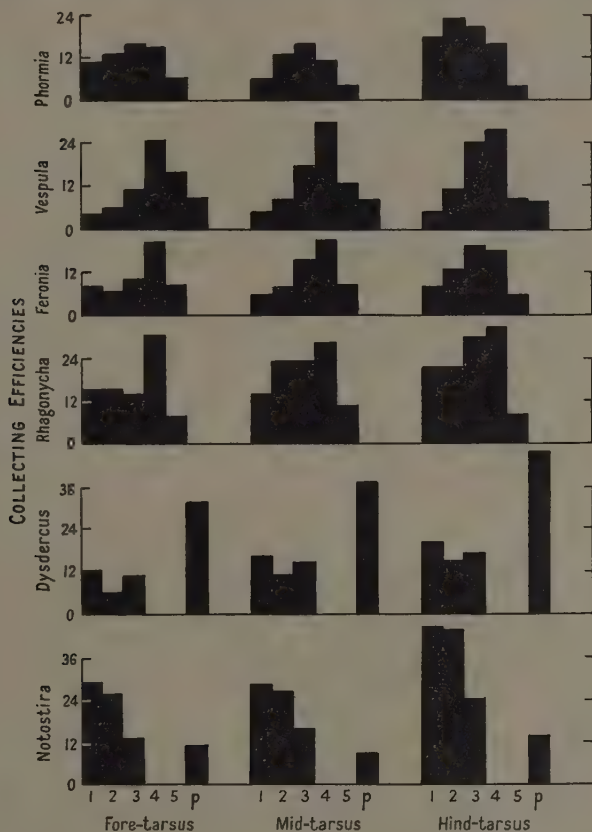


Fig. 4.—The average collecting efficiencies of the tarsal segments and the pretarsal lobes.

species have a low efficiency, probably because they rarely touch the substrate. The proximal part of each fifth segment is shielded from the substrate by the lobes of the fourth segment and the distal part may be held clear of a flat surface by the presence of the claws. The particles acquired by the fifth segments may be transferred to them from the lobes of the fourth segment and not picked up directly from the substrate.

Three-segmented tarsi.—There is a marked difference in the histograms for *Dysdercus* and *Notostira*, the two species with three-segmented tarsi which were studied. Because only the tips of the spines of the second tarsal segments of *Dysdercus* brush the surface their uptake is small, whereas the corresponding

segments of *Notostira* have a much larger area of contact with the surface and, consequently, a much higher uptake per unit length. A further difference is that the thrust by the pretarsal lobes of *Dysdercus* is concentrated into a small area of contact with the surface, so that they are very highly efficient accumulators of particles, unlike those of *Notostira*. These two differences are largely responsible for the differences in the histograms for the collecting efficiencies of the two species.

The importance of tarsal setae in the uptake of particles.

From observations on the position of particles picked up by the tarsi it was concluded that, in all the insects studied, particles are picked up by the tarsal setae, chiefly the ventral ones, by the claws and by the pretarsal lobes, where these are present, and that, except in the case of *Feronia*, particles only reach the main surface of the cuticle indirectly. Where the tarsal setae are dense, as in *Vespula* and *Rhagonycha*, particles do not reach the main surface of the cuticle easily, although the cleaning movements of *Vespula* were found to facilitate the process. Where only a few setae are present, as in *Feronia*, the main part of the cuticle is quickly contaminated, particles probably being picked up directly upon the latter.

The Effect of Redistribution and Loss of Particles on the Proportions present on different Regions of Wasps.

In addition to the study of the distribution of particles on the legs of insects leaving a dusted surface, an investigation was made of the distribution of particles on contaminated insects which had been left on a clean surface for varying lengths of time after exposure. Wasps (workers of *V. vulgaris*) were selected for this study of redistribution and loss of particles because they exhibit marked cleaning behaviour, which is an important factor in the redistribution of particles on contaminated insects and might therefore alter the proportion of material on several parts of the body.

Estimations by the colorimetric method of the proportions of lipophilic dyestuff present on the head, the thorax (including the wings and the legs) and the abdomen of contaminated wasps, which had been allowed to clean and to walk about on a clean surface for varying periods of time up to one hour, showed that the percentage of the total dyestuff on the insect on the three main regions of the body does not vary with the time since exposure (Table III).

TABLE III.

The proportions of particles present on the head, thorax and abdomen of contaminated wasps at varying times after exposure.

Time (min.) since exposure	Per cent. on head	Per cent. on thorax	Per cent. on abdomen
0	8	68	24
5	9	68	23
10	12	70	18
20	7	71	22
60	11	67	22

Each set of figures is the average for four wasps.

Therefore particles are lost from the head, thorax and abdomen in the proportions in which they are acquired and cleaning movements transfer particles in constant proportions from one region to another. A similar result was obtained by Lewis & Hughes (1957) with blowflies. Since there was no change in the proportions of dust on the main regions of the body of these insects which clean themselves vigorously, it was considered improbable that there would be any change in insects which show less marked cleaning reactions.

The ratio of the amounts of dyestuff on the different pairs of legs also did not vary significantly (at the 5% level of probability) with the length of time since exposure of the insects to a dusted surface (Table IV).

TABLE IV.

The proportions of particles present on the fore-, middle and hind legs of contaminated wasps at varying times after exposure.

Time (min.) since exposure	Percentage of total amount of dust on legs		
	Fore	Middle	Hind
0 	20	30	50
5 	19	34	47
10 	27	21	52
20 	14	30	56
40 	15	32	53
60 	22	27	51

Each set of figures is the average for four wasps.

Because of the presence of an antennal cleaning comb on each fore-tarsus the lack of increase in the proportion of material on the forelegs was unexpected but might be explained by the formation of large clumps of particles, which would be sufficiently heavy to drop off the cleaning combs.

The particles were counted on each tarsal segment of four wasps one hour after transferring them from a dusted to a clean surface. A comparison of the distribution of the particles on these wasps with that on wasps examined directly after removal from a dusted surface showed (Table V) that cleaning and loss of particles causes (1) a marked decrease in the proportion of dust on those segments which collect particles most efficiently (see fig. 4), namely, the fourth of all the tarsi and the third of the hind tarsi, and (2) a marked increase in the proportion on those segments which have the lowest collecting efficiencies (see fig. 4), namely, the first of all the tarsi and the second of the fore-tarsi.

This change in the proportions of the dust on the different tarsal segments reduced the variation in the quantities per unit length, bringing about a more uniform distribution of particles along the length of each tarsus. The relatively higher rate of loss from the segments with the greatest concentration of particles might be accounted for by the formation of clumps of particles, which would be more easily detached, or by the action of cleaning movements.

Discussion.

Factors determining the uptake of particles.

Theoretical considerations suggest that the amount of material picked up by an insect from a deposit of dust depends on the *true area of contact* of the insect

with the substrate. The true area of contact may be defined as the sum of the areas of the apposing surfaces. This area is determined by the force applied to the *apparent area of contact*, which is the area over which cuticle and cuticular outgrowths touch the substrate. Because the setae articulate with the main surface of the cuticle, an increase in the thrust increases the area of the setae which is in contact with the substrate and setae which otherwise would not

TABLE V.

The distribution of particles on wasp tarsi on removal from a deposit and one hour later.

Percentage of particles on each segment*				
			On leaving deposit	One hour after leaving deposit
Fore-tarsus				
Segment 1	23	44
2	8	15
3	12	10
4	30	14
5	18	11
pretarsus	9	6
Middle tarsus				
Segment 1	19	40
2	12	13
3	20	15
4	32	15
5	11	11
pretarsus	6	6
Hind tarsus				
Segment 1	21	48
2	16	18
3	26	13
4	26	12
5	7	7
pretarsus	4	2

* The number of particles on each segment is expressed as a percentage of the total on the tarsus of which the segment forms a part.

The figures for the wasps on leaving a deposit are the average for eight insects and those for wasps one hour later are average for four insects.

touch the substrate are brought into contact with it. The apparent area of contact is determined by the size and structure of the insect, in particular of the tarsi, which are the chief sites of uptake, and the manner in which the insect walks, while the force applied to the substrate depends on the weight of the insect. The actual pressure on the substrate, however, varies with the area over which the weight is distributed. Because of these complex inter-relationships it is difficult to assess the relative importance of the various factors involved in the uptake of particles.

The small variation in the ratio of the quantities of dust picked up by the different pairs of legs in insects of varying size and structure indicates that there is a fundamental pattern for the ratio of the uptake between the different pairs of legs which is common to all species walking on six legs. Since this pattern cannot be correlated with differences in the structure of the three pairs of legs, it is presumably determined by the relative positions of the legs and their different functions in locomotion.

In addition to picking up more particles than the fore- or middle tarsi, the hind tarsi pick up more particles per unit length. This greater importance of the hind tarsi is probably due to the large thrust which they exert in association with their propulsive function.

The distribution of the particles picked up on the tarsi differs from one species to another. This difference is thought to depend on the position of the centre of thrust and the regions which touch the substrate.

The effect of tarsal structure on the effectiveness of a dust insecticide.

Penetration of insecticide through the main surface of the cuticle is likely to be greater and nearer to the site of action than penetration into the tarsal setae. Therefore, in the absence of evidence that hairy tarsi (*i.e.*, those that are densely clothed with setae) will pick up more particles than tarsi with fewer setae, the same insecticidal treatment is likely to be less effective on hairy insects than on less hairy ones, since a dense covering of fine tarsal setae is likely to protect the main surface of the cuticle by helping to keep particles away from it. David & Gardiner (1950) found that the progressive increase in hairiness of insects is paralleled by increase in resistance to DDT dusts, which they suggested was due to the hairs forming barriers keeping the DDT from sites where penetration usually occurs.

Fine setae will, however, only succeed in decreasing the effectiveness of an insecticidal dust in the absence of cleaning behaviour, for during cleaning movements particles are liable to be transferred from the tips of the setae to the main surface of the cuticle. Thus the main surface of the cuticle will become contaminated by this process and the setae will be left free to pick up more particles from the deposit. Therefore, an insecticidal treatment is likely to be more effective on an insect which cleans frequently than on one which cleans rarely, and in the case of a hairy insect the effect of cleaning may counteract any protection afforded by a dense covering of tarsal setae.

Although the uptake of particles by the pretarsi is comparatively small and those pretarsi which possess pulvilli or arolia do not pick up a higher proportion of particles than those which lack them, the uptake of insecticidal particles by pretarsal lobes is not necessarily unimportant. Work by Slifer (1950) suggests that the arolia of ACRIDIDAE, particularly after they have been worn or injured, are areas through which insecticides may enter with relative ease. In the case of a nerve poison, the insecticide which has penetrated the cuticle at these points will be close to a site of action because these arolia contain nerves, unlike the pulvilli of blowflies (Lewis, 1954a). Therefore, although the quantity of insecticide picked up by the pretarsi of insects of different species may not vary with structure, the presence of pretarsal lobes may increase the rate of penetration of the amount picked up while the presence or absence of pretarsal nerves may determine its effectiveness.

Summary.

The quantities of lipophilic dyestuff picked up by specimens of five insect species, namely *Vespula vulgaris* (L.), *Feronia madida* (F.), *Rhagonycha fulva* (Scop.), *Dysdercus fasciatus* Sign. and *Notostira erratica* (L.), in their first few steps on a dust deposit of the dyestuff have been estimated. The uptakes per step by insects of different size and structure were shown to differ only slightly. While the uptake was found to be greater for the larger insects, the smaller insects picked up more particles per unit body weight at each step.

The ratios of the actual amounts (estimated by a colorimetric method) picked up by the different pairs of legs are similar for all the insects that have been studied and in all cases the hind legs were shown to pick up an amount at least equal to the sum picked up by the fore- and middle legs.

The distribution of particles picked up by the tarsi of the five species of insects has been determined by counting, under a microscope, all the particles on representative parts of the tarsal segments. The results, together with those for a sixth species, *Phormia (Protophormia) terraenovae* R.-D., quoted from a published work, are given. The first segments of the hind tarsi of four of these species pick up considerably more than any of the other tarsal segments. The pretarsi of all legs accumulate only a small percentage of the total uptake. The hind tarsi as a whole are shown to be more important accumulators of particles than the fore- or the middle tarsi. The measure of the efficiency with which each tarsal segment collects particles (the collecting efficiency) used here is the percentage uptake per unit length of segment. The significance of the differences in these efficiencies is discussed. It is concluded that the collecting efficiency of a segment is partly a function of the thrust applied to the substrate and, therefore, that measurements of the uptake of particles should provide information concerning the method of use of the tarsal segments in walking, *i.e.*, the degree of thrust applied by those regions of the tarsus which touch the substrate.

From observations on the position of particles picked up by the tarsal segments it is concluded that the tarsal setae help to keep particles away from the main surface of the cuticle, which is not easily contaminated where the setae are dense.

Investigations of the effect of redistribution and loss of particles on an insect which performs vigorous cleaning activity have shown that particles are lost from head, thorax and abdomen in the same proportions in which they are acquired and that cleaning movements transfer particles in constant proportions from one region to another. The proportions of particles on the three pairs of legs also remain constant but the distribution on the tarsal segments changes.

The influence of structure and behaviour on the contamination of different insects is discussed. It is concluded that an insecticidal treatment is likely to be less effective on insects possessing tarsi that are densely clothed with setae than on those with fewer tarsal setae and more effective on insects that clean themselves frequently than on those that do so rarely. The effect of cleaning by insects that are densely clothed with setae may counteract any protection afforded by these setae.

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STUDIES CONCERNING THE UPTAKE OF CONTACT INSECTICIDES.

II.—THE CONTAMINATION OF FLIES EXPOSED TO PARTICULATE DEPOSITS.

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A survey of the literature reveals few references to the precise sites of uptake of particles on the tarsi and pretarsi, the effects of "cleaning" reflex movements on the location and retention of particles and the influence of lipid solubility (a fundamental property of contact insecticides) on the adhesion of dry particles to the lipophilic surfaces of insect and leaf cuticle. It has been assumed that the pulvilli are important sites of contamination when species of Diptera are exposed to deposits (Potts & Vanderplank, 1945; Hayes & Liu, 1947; Sarkaria & Patton, 1949), but no data have been published to support this view. Barlow & Hadaway (1952) have determined the weights of crystals retained by adults of *Aedes aegypti* (L.) at various times after exposure to DDT deposits. Crystals were found to be slowly lost from the exterior of contaminated mosquitos at rates which varied with particle size. The authors considered that particles smaller than 20 microns in size were lost largely by absorption through the cuticle while bigger particles were lost largely by detachment.

The ingestion of dust particles from deposits following cleaning reactions between the tarsi and mouthparts in species of Coleoptera and Orthoptera has been observed by Mote, Wilcox & Davis (1926), Richardson & Glover (1932) and Smith (1938).

This paper presents the results of an investigation of certain processes taking place when flies of the species *Phormia* (*Protophormia*) *terraenovae* R.-D. are exposed to finely particulate deposits.

Materials and Techniques.

The availability of insecticidal deposits to exposed insects is known to vary considerably with formulation and substrate. The subject has been reviewed by Hadaway & Barlow (1952). To eliminate some of the many variables, deposits of non-irritant particles of constant mass median diameter were used in the present study.

Deposits were produced by a method which combined particle-size separation by air sedimentation with the provision of an even deposit. Particles were ejected by a brief compressed-air blast from an earthed copper tube, suspended centrally inside a bell jar placed over an open tower seven ft. in height, and were allowed to settle in the form of a dust cloud. The larger and most of the smaller particles settled out first from dust clouds, the smaller particles (especially those of diameter $<3\mu$) in aggregates because their very large surface area/weight ratio caused them to adhere in clumps (*cf.* Gregg, 1951). By exposing surfaces at the base of the tower after a lapse of 2 minutes from the time of ejection of dust samples it was found possible to obtain, repeatedly, deposits of particle size $20\mu \pm 3$ mass median diameter. Such deposits were used in all experiments. Particle sizes in samples of deposits were measured, using a microscope fitted with a micrometer eyepiece. An example of the particle-size distribution in a typical deposit is given in fig. 1.

The density and uniformity of the deposits obtained were checked by measuring the quantities deposited on small coverslips, 2.5 sq. cm. in area, that were distributed on the dusting platform at the base of the tower around the larger surfaces, of 50 sq. cm. area, required for experiments.

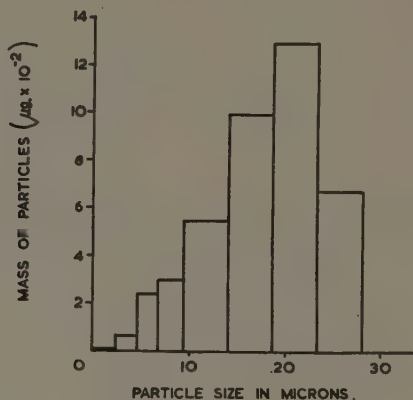


Fig. 1.—The particle-size distribution of a typical deposit.

Particles of pure dyestuffs were used for the deposits employed in most experiments and quantities were determined colorimetrically. By this means many assessments of small quantities of particles adhering to flies could be obtained quickly and accurately.

The substances chosen were BDH oil-soluble violet and Casella diamine aldehyde blue. The latter was relatively insoluble in lipoids and fat solvents. For example, the respective solubilities of the two dyes at 25°C. were 12.5 and less than 1×10^{-4} grammes per litre in xylene, 55 and 4×10^{-3} g./l. in acetone and 1×10^{-2} and 25 g./l. in water. Throughout this account the dyestuffs will be termed lipophilic and lipophobic, respectively. Particles of both substances were approximately isometric. The two dyestuffs were selected primarily to show up differences in adhesion due to lipid solubility. It is not suggested that they provide an exact parallel in adhesive behaviour to contact-insecticide and diluent dusts, though these are also lipid-soluble and insoluble respectively.

Blowflies of the species *P. terraenovae* were reared in a constant temperature room at 25°C. and 70 per cent. relative humidity. The larvae were cultured in small batches of 50 to 100 on raw beef. Adult flies weighing 42 ± 4 mg. were used in experiments. In the majority of experiments, flies were exposed to treated surfaces for short intervals of time, in a constant temperature room at 25°C. and under a very bright light (100 watts at a distance of 2 ft.). In these conditions of temperature and illumination, small batches of flies walked continually at a steady average rate; this was demonstrated by the good agreement of successive measurements of the uptake of particles from standard deposits by walking flies (as, for example, the results presented in fig. 5).

The exposure apparatus consisted of a shallow perspex chamber measuring $7 \times 7 \times 0.8$ cm. internally, with a detachable lid in which was incorporated a baffle device for the distribution of carbon dioxide gas (fig. 2). Clean or treated surfaces were slipped in and out of the apparatus as required through slots at the base of the chamber. With a surface in position, the height of the chamber was

such that the flies were able to walk freely without touching the top, but were restricted from turning over to walk on the lid.

The following experimental procedure was adopted:—

- (1) A batch of eight flies, anaesthetised with carbon dioxide, was placed in the exposure apparatus on a clean paper surface.

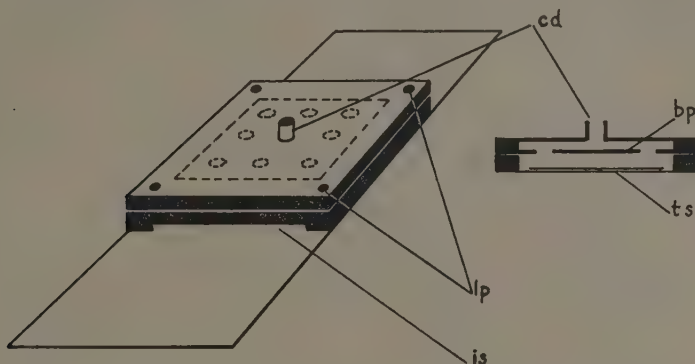


Fig. 2.—The apparatus for exposing flies to deposits: bp, baffle plate; cd, carbon-dioxide duct; is, slot for insertion of treated surfaces; lp, locating pins for lid of chamber; ts, treated surface.

- (2) Ten minutes after the flies had recovered, the clean surface was replaced by a treated surface, inserted for a timed interval.
- (3) The treated surface was removed and the flies gassed immediately with carbon dioxide. (Alternatively, for experiments on the cleaning of contaminated flies, a clean surface was exposed at this stage.)
- (4) Flies were transferred to a measured volume of solvent (acetone or water). The intensity of colour developed was measured by a Spekker adsorptiometer, and from this measurement the weight of particles taken up by the eight flies was calculated.

The measurement of quantities picked up in the act of settling was made difficult by the excitability of flies at 25°C. Such measurements were therefore best made at 14°C., at which temperature activity was depressed. Using bees-wax applied to the dorsal surface of the thorax, flies were attached by lengths of fine wire to the objective block of a dissecting microscope so that the flies were in focus when viewed through the instrument. The fine wire allowed the flies considerable freedom of movement. When the microscope was racked down, an active fly was steadily moved towards a treated surface lying in an open beaker in an atmosphere of carbon dioxide. Under close observation the fly was seen to make a normal settling action, or it was discarded. As it became stupefied, either without further movement or after taking two or three steps, the fly was withdrawn from the surface without struggling by racking up the microscope. The quantity of particles adhering to the fly was then estimated colorimetrically.

Contamination of the Tarsi.

Rate of accumulation.

The weights of particles picked up were determined when continuously active flies were exposed to standard deposits for periods varying from 30 seconds to 4 minutes. The deposits were of lipophilic or lipophobic particles on waxy or

fibrous surfaces. The rate of uptake was always found to have decreased rapidly during the first minute (fig. 5).

Experiments with flies alighting on deposits under controlled conditions, as described above, further showed that the quantities of particles picked up in the act of settling considerably exceeded the quantities picked up in subsequent steps. For example, it was deduced that after only 1 second, or 6 footsteps, flies picked up particles at about 25 per cent. of the initial rate (fig. 3).

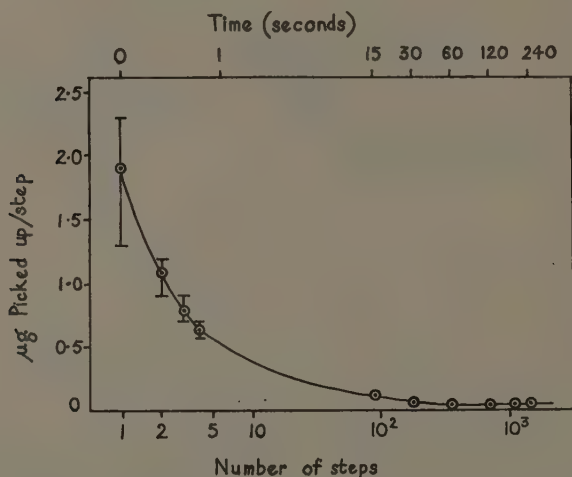


Fig. 3.—Weight of particles picked up per step at various stages during exposure of adults of *Phormia* to a deposit of lipophilic particles on filter paper. Deposit density $27.5 \mu\text{g. per sq. cm.}$, mass median diameter of particles 20 ± 3 microns. One step was defined as one movement of all six legs; the rate of movement was 6 steps per second.

These observations are readily explained by the fact that when a fly makes its first contact with a treated surface all the tarsal spines can take up particles. As the more favourable reception points on the tarsi become contaminated, the rate of uptake is necessarily diminished.

Immediate sites of uptake.

Flies were exposed for very short periods (3 to 6 seconds) to deposits of dry crystals of salicyldiazine, of particle size 20 ± 3 microns mass median diameter. The yellow crystals of this substance showed up clearly against the black tarsal integument. The tarsi were removed before the flies performed "cleaning" movements and the numbers and precise locations of particles were determined by examination under a microscope at $\times 400$ magnification.

It was observed that the majority of particles were picked up at the tips of the ventral tarsal setae but some were to be found adhering away from the tips. By means of a low power microscope aligned horizontally with a treated surface, observations were made of the behaviour of the ventral spines of chilled flies that were slowly performing walking movements. It was disclosed that occasionally at impaction and less frequently at levation the ventral spines were momentarily splayed outwards, approximating to the surface along about half the length of each spine; the spines then immediately retracted to the normal position, making

contact with the surface only at the tips. The occurrence of particles contaminating parts of the ventral spines remote from the tips was thus explained. The displacement of the spines was due to the elasticity of the articulating membranes, the spines always remaining rigid.

Frequently, particles from the deposit adhered to other particles previously picked up by the tarsal spines and formed aggregations. Such aggregations were to be found on the tarsi at all times during exposure. The clumps of fine particles behaved as large particles and were continually discarded and re-formed.

The numerous setae which cover the five tarsal segments of adults of *P. terraenovae* are distributed in a clearly defined pattern (Lewis, 1954a). Long spines situated at the distal ventral extremity of each segment are the principal points of contact with which a fly engages the substrate, being longer than the remaining tarsal setae. The heaviest accumulation of particles is invariably upon these primary spines which exert greater pressure on the substrate than any other part of the tarsi. The intersegmental joint membranes, situated immediately above the contaminated extremities of the primary spines, remain surprisingly free from particles. Extending along the full length of each segment are two ventral rows of flexible contact chemoreceptors which touch the substrate and receive particles on the distal parts of the frontal membranes. These delicate tarsal chemoreceptors, of which there are more than one thousand on each fly, are protected from excessive pressure against irregularities of the substrate by adjacent rows of rigid secondary spines which restrict the uptake of particles by the chemoreceptors. The total quantity of particles picked up by the numerous secondary spines exceeds that acquired by the primary spines although the density of contamination is greater on the latter.

Collecting efficiency of different tarsal segments.

When the degrees of contamination of the different tarsal segments were compared by counting all the attached particles, it was discovered that on all legs the segments nearest the body picked up substantially more particles than the distal segments and pretarsi (Table I). The first tarsal segments collected

TABLE I.

Percentage distribution of particles of salicyldiazine on the tarsi before the initiation of cleaning-reflex movements.

Segment	Fore-tarsus		Middle tarsus		Hind tarsus	
	Uptake (%)	Collecting efficiency	Uptake (%)	Collecting efficiency	Uptake (%)	Collecting efficiency
1st ..	9	10.2	6	5.4	20	17.4
2nd ..	5	13.2	6	12.5	11	23.0
3rd ..	5.5	15.8	5.5	15.3	8.5	20.7
4th ..	4	14.8	3.5	10.9	5	15.6
5th ..	2	6.2	1.5	3.9	1.5	3.8
Total ..	25.5	11.6	22.5	8.5	46.0	16.4
Pretarsus	2.5		2.0		1.5	

Average for 5 flies exposed for 3 to 6 seconds. The collecting efficiency of a segment is expressed as the percentage uptake per unit length in mm.

35 per cent. of the total taken up by the tarsi, whilst the pretarsi, including the pulvilli, picked up only 6 per cent.

The tarsal segments vary considerably in length and differences in uptake are clearly related in part to these differences in length. When allowance is made

for this factor by translating values for the absolute uptake per segment to uptake per unit length, it can be seen that differences in the collecting efficiencies of segments exist which are independent of segment length (Table I). For example, although the long first tarsal segments picked up most particles they were not as efficient as the middle segments.

The segmental variations in collecting efficiency can be attributed either to differences in structure or to the thrusts with which segments engage the substrate. Three of the fifteen different segments exhibit a relatively high collecting efficiency by reason of a variation of the typical setal pattern. These are the first segments of the fore-tarsi and the first and second segments of the hind tarsi. They possess a considerably greater density of ventral setae than, for example, the corresponding segments of the middle legs and this structural difference is reflected by the number of particles picked up. However, no morphological explanation can be found for the variations in uptake per unit length among the remaining segments. It is therefore suggested that the setae of certain segments are pressed more firmly against the contaminated substrate than are those of other segments and that differences in the collecting efficiencies of structurally similar segments are an indication of the thrusts exerted upon different segments in the act of walking.

If this hypothesis is correct, the results given in Table I appear to indicate that, after allowing for the high density of ventral bristles on the proximal segments, the hind tarsi exert the greatest thrust on a deposit in the act of walking and are therefore the principal propulsive organs. It also appears that the middle segments of each tarsus engage the substrate more completely than the distal and proximal segments. The fact that the contact chemoreceptors are more closely packed on these middle segments (Lewis, 1954a) provides an interesting correlation of form and function. The greater density of chemoreceptors does not in itself provide an explanation of the greater collecting efficiency of the middle segments, for though the second and third segments of the fore-tarsi possess 30 to 40 per cent. more receptors than the corresponding segments of the middle tarsi, the collecting efficiencies differ only by 5 per cent.

Effect of Cleaning Movements on Distribution.

The ventral sides of the tarsi, with their combs of spines, are not only the principal sites of uptake but are also the cleaning instruments. A contaminated fly soon begins to perform cleaning movements, usually within 30 seconds of exposure. The forelegs are rubbed together and on the sides of the head; the middle legs are cleaned by the forelegs; the hind legs rake the sides of the abdomen, the leading edges of the wings and they are also rubbed together.

TABLE II.

Distribution of particles on contaminated adults of *Phormia* at all times after the initiation of cleaning-reflex movements.

Part of insect	Percentage, by weight		
Antennae	0.5	to	1
Rest of head	6	±	1
Wings	13	±	2
Forelegs	13	±	2
Middle legs	13	±	2
Hind legs	22	±	2
Abdomen	30	±	5
Thorax, less appendages	2	±	1

These movements transfer particles from the distal parts of the ventral tarsal setae to the hitherto uncontaminated microtrichia of the main tarsal cuticle, to the basal parts of the setae and to the head, wings and abdomen. The cleaning movements take place frequently, and thus proportions of the particles taken up are constantly being transferred to a variety of parts of the fly previously unexposed to contact. Few particles of the size used in these experiments are lost in the transfer.

Many determinations of the percentage, by weight, accumulated at different locations on the body were made and the results were always the same, however much the total dosage, duration of exposure or duration of cleaning after treatment were varied (Table II).

The stereotyped cleaning movements therefore act as an equilibrating mechanism, providing a continual adjustment of the quantities associated with various parts of the body during exposure to a deposit. When the insect is no longer exposed to the deposit, the equilibrating influence of cleaning movements continues to be effective throughout the period in which particles are being discarded by the fly.

Loss of Particles from Contaminated Flies.

The efficiency with which contaminated flies were able to rid themselves of particles was next investigated.

Flies were allowed to pick up known amounts of dry lipophilic particles and then exposed to clean surfaces. The weights of dust lost over various periods of time were estimated by determining the weight remaining on the flies and the weight transferred to the new surfaces.

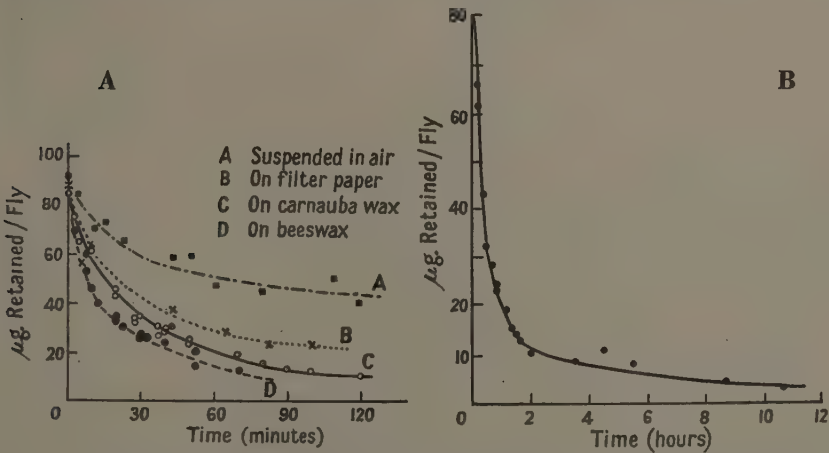


Fig. 4.—The discarding of lipophilic particles by active contaminated adults of *Phormia*: A, a comparison of rates of loss on various clean surfaces or suspended in air; B, the rate of loss on a carnauba leaf wax surface over longer periods.

Results are illustrated by fig. 4, which shows that particles were discarded most readily on wax surfaces (curves C & D). The fact that the rate of loss varies with substrate suggests that a significant proportion of the discarded particles must be lost by way of the points of contact between the tarsi and the substrate.

Other batches of treated flies were suspended in air from fine wires, when they

were able to perform cleaning movements without making contact with a surface. These flies lost their particles at about one-third of the rate at which those on surfaces of carnauba palm-leaf wax (produced by *Copernicia cerifera*) lost theirs, when cleaning movements were performed at approximately the same frequency. It was concluded that about two-thirds of the discarded particles were "walked" off at the tarsal spines, by the reverse of the "pick-up" process. As particles were lost from the tarsal spines they were replaced by others, recovered by cleaning, from other parts of the body.

Experiments also showed that the rate of loss at any given dosage on a fly was independent of the way in which the fly acquired that dosage. The particles were discarded at the same rate, corresponding to the appropriate region of the graphs in fig. 4, whether they had been acquired during continuous or intermittent exposures to deposits, or whether they formed the residue from a higher original dose which had been partly cleaned off. Examples are given in Table III. This

TABLE III.

A comparison of the rates at which lipophilic particles were discarded on clean carnauba-wax substrates by active *Phormia* which had originally picked up different quantities.

Initial dose received ($\mu\text{g./fly}$)	Rate of loss at different contamination levels ($\mu\text{g./minute}$)		
	50 $\mu\text{g./fly}$	35 $\mu\text{g./fly}$	25 $\mu\text{g./fly}$
84	1.40	0.68	0.43
70	1.35	0.74	0.37
62	1.41	0.72	0.41
35	—	0.70	0.43

The rates of loss were derived graphically from the slopes of retention-time curves (*cf.* fig. 4).

finding could have been forecast from the observations on the equilibrating effect of cleaning movements.

The rate of loss on a wax substrate (fig. 4) was relatively rapid until the quantity retained by a fly had fallen to about 20 $\mu\text{g.}$ and was very slow below 10 $\mu\text{g.}$ After two hours' cleaning, the weight retained by an active insect was of the same order for a wide range of original doses (Table IV). Thus, although

TABLE IV.

The discarding of particles by active adults of *Phormia* on a clean carnauba-wax substrate, at different levels of contamination.

Original dose on fly ($\mu\text{g./fly}$)	Quantity retained after 2 hr. ($\mu\text{g./fly}$)
80	11
40	10
20	8.5
10	7

10 $\mu\text{g.}$ of particles can be accumulated by an active fly within two or three seconds on a dusted surface, much of the contamination will persist after two hours of continuous activity, owing to the transference of particles away from the points of the ventral tarsal setae, brought about by the cleaning movements. It should be observed that two hours of continual movement induced during these experiments by a high light intensity at 25°C. probably produced an effect equal to that of many hours of normal activity on the part of a blowfly.

The Effect of Lipoid Solubility on the Adhesion of Particles to Lipoid Surfaces.

Uptake of particles from filter paper.

Flies were exposed to deposits of lipophilic or lipophobic particles of particle size $20 \pm 3 \mu \text{ M.M.D.}$ and deposit density $27.5 \mu\text{g./sq. cm.}$ under standard conditions of temperature and illumination as described previously. From Whatman No. 1 filter-paper surfaces, the lipophilic particles were picked up at an appreciably faster rate than were the other particles (fig. 5). For example, after two

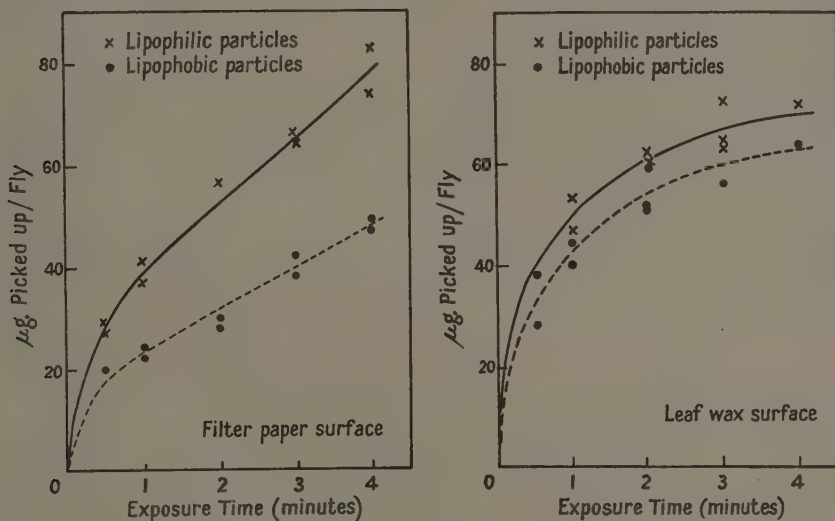


Fig. 5.—The cumulative uptake of dry particles by active adults of *Phormia* from deposits on filter paper and carnauba leaf wax. Deposit density $27.5 \mu\text{g. per sq. cm.}$, mass median diameter of particles $20 \pm 3 \text{ microns.}$

minutes' exposure, average weights of $53 \mu\text{g.}$ of lipophilic particles and $31 \mu\text{g.}$ of lipophobic particles had been taken up per fly.

The fact that the lipophilic particles were picked up more than half as fast again as the lipophobic particles from otherwise similar deposits indicates that the oil-soluble particles adhered more readily to the lipid epicuticle of the flies than did the water-soluble particles.

Availability on waxy surfaces.

When the experiment was repeated, using surfaces which had been coated with a smooth deposit of a hard leaf wax (carnauba wax, M.P. 84°C.), the difference between the rates of accumulation of the two types of particles was much less than that observed for deposits on filter paper (fig. 5). The lipophilic particles were 1.67 times more available to flies than were the lipophobic particles when deposits on filter paper were compared, but only 1.2 times more available

on the leaf wax. This finding suggests that the lipophilic particles are less easily detached from the wax substrate than from the fibrous paper; *i.e.*, while the lipophobic particles are relatively indifferent to lipid surfaces, the lipophilic particles have an affinity for both the lipid epicuticle of the insects and for the leaf wax.

The *absolute* quantities picked up on the two surfaces cannot be compared because the physical textures of the two types of surface are different. A considerable proportion of a deposit on filter paper lies in the inter-fibrillar spaces out of reach of a walking insect; thus the available effective deposit was of a higher density on a wax surface though the nominal deposit density was the same. This fact does not invalidate the comparison of the *relative* rates of accumulation as given above.

The experiment was repeated a third time, using surfaces coated with beeswax. The quantities of particles taken up by flies from this softer wax surface were very variable (fig. 6) but consistently smaller than the quantities of particles taken up from carnauba wax; both types of particle were held more firmly by the softer wax.

Availability of oily particles.

As a sequel to experiments on the adhesion of particles to hard and soft wax surfaces, the availability of particles associated with an oil film was investigated. Flies were exposed to deposits of lipophilic particles on filter paper treated with a mineral oil, Shell Risella 17, at the rate of 0.2 ml. per 100 sq. cm. The particles were wetted by the oil, but the rate of solution was found to be negligibly slow.

Over a range of deposit densities, the oily particles were taken up at only 10 per cent. of the rate at which equivalent deposits of dry particles were picked up (fig. 6). The oily particles adhered tenaciously to the substrate although the tarsal spines were wetted by the oil.

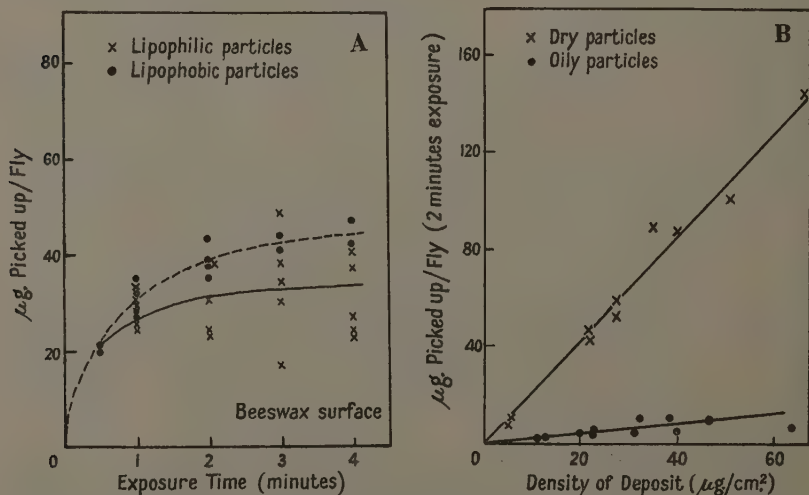


Fig. 6.—The cumulative uptake of particles by active adults of *Phormia*: A, uptake of dry particles from beeswax surfaces. Deposit density 27.5 $\mu\text{g.}$ per sq. cm.; B, uptake of dry and oily lipophilic particles from filter paper by flies exposed for two minutes to deposits of different densities. Mass median diameter of particles 20 ± 3 microns.

Discussion.

Short exposures.

The results described above show that the collecting efficiency of a blowfly exposed to a loose particulate deposit diminishes rapidly; *e.g.*, by about 30 per cent. immediately after the initial contact and by 90 per cent. after 15 seconds of continuous walking over a light deposit. It seems that if a deposit is of fine, detachable particles, a very brief contact can furnish a "hairy" insect (*i.e.*, one that is densely clothed with setae) with an appreciable dose, a proportion of which will persist for many hours provided the insect performs cleaning movements to redistribute the contaminating particles over its body. This result may help to explain the fact that insecticidal deposits can be effective against insects such as mosquitos which do not walk freely after settling and may be repelled into flight after a brief contact with a chlorinated hydrocarbon insecticide (Kennedy, 1947; Muirhead-Thomson, 1950; Wharton, 1951; Hadaway & Barlow, 1953).

Cleaning movements.

If it were not the habit of contaminated insects to perform cleaning movements, it is certain that dry deposits of contact insecticides would be generally far less effective. The uptake of particles would almost cease after the points of contact of the ventral tarsal setae had been covered, and the dose so acquired would be readily discharged from the points when the insect moved to a clean surface. The cleaning movements facilitate a higher rate of uptake by continually decontaminating the tarsal points of contact between insect and deposit, and transfer material in constant proportions to other parts of the body, previously unexposed to direct contact with the insecticide, but that are in some cases more vulnerable (Table II). Particular interest attaches to the 7 per cent. received by the head, for it has been found that the head is the region most susceptible to DDT in *Musca* (Fisher, 1952; LeRoux & Morrison, 1954) and in *Phormia* (Lewis, 1954b).

The discarding of particles.

No evidence was found for the belief that particles may lodge between setae and remain wedged in place. It follows that there is no reason to suppose that the optimum particle size of a deposit will vary for different insect species.

A particle, transiently caught between a surface and the tip of a tarsal seta, will adhere to that which touches it over the greater surface area (neglecting for the moment the question of interfacial affinities, discussed below). Thus particles may be transferred from the substrate to a seta or *vice versa*, and a contaminated fly will lose particles to a clean surface by the same process by which it picked up particles from a treated surface. The rate at which particles are discarded by a fly to a clean surface by this means will depend on the rate at which particles are "cleaned" back from other parts of the body to the tips of the ventral setae. As the particles retained by a fly become fewer, the chance of a tarsal seta receiving a particle in a position suitable for discharge becomes less. Theoretically therefore the dose retained by an active insect should approach zero at an ever decreasing rate. The experimental data is in close agreement with this argument (fig. 4).

The above discussion applies only to fine particles which have a high surface area/volume ratio, when the forces of adhesion exceed the particle weight. Larger particles, possessing a weight of the same order as the adhesive forces, will be knocked off in cleaning movements.

It has been found that some particles are lost even when a suspended fly cleans itself in space. This loss is explained by the gradual aggregation of fine particles into large clumps, which then behave as large particles which can be knocked off.

Thus there are two mechanisms by which flies discard fine particles: (1) by "walking" them off from the tarsal spines, (2) by forming them into aggregates and knocking off the clumps. The former has been found to be the more important mechanism when adults of *P. terraenovae* are contaminated with dry non-irritant particles. If the contamination had been of irritant insecticide particles, it is possible that both discarding processes might have been executed at a faster rate.

Lipoid solubility and the adhesion of particles to surfaces.

It has been demonstrated that lipid-soluble particles adhere more easily to blowfly epicuticle and to a leaf wax than do lipophobic particles. The adhesion of a lipophilic particle to a lipid surface presumably depends upon the van der Waals forces at the solid/solid interface. Polar groups, possessed by the lipophobic particles used in the above experiments and by "inert" diluent dusts such as calcium or magnesium silicate, would certainly reduce attractive forces of this kind at a lipid surface. Thus the forces of adhesion to the insect cuticle should be stronger for lipid-soluble particles than for inorganic or water-soluble organic substances. Adsorption of water molecules may also play an important part in phenomena of adhesion. Particles with exposed polar groups can adsorb more water from the atmosphere than particles which are hydrophobic and lipid-soluble, and such adsorbed moisture would reduce the adhesive forces at a lipid surface.

On a soft-wax substrate a particle may be pressed slightly into the surface when touched by the rigid spines of an insect tarsus. The resultant increase in contact area between particle and substrate must increase the adhesive force between them, which is proportional to contact area. This pressure effect influences both types of particle equally and is well illustrated by measurements which were taken of the rates of accumulation from a soft beeswax surface (fig. 6). Both types of particle were held more firmly by the beeswax than by the harder carnauba wax (fig. 5).

Oily particulate deposits.

It has been shown that oily particles are picked up by walking flies from a deposit at about one-tenth of the rate at which dry particles are accumulated. Yet oily crystalline deposits of DDT are much more toxic to mosquitos than are dry crystalline deposits obtained by the evaporation of a solution in a volatile solvent. (Elmendorf & others, 1946; Barlow & Hadaway, 1950.) It thus appears that the greater toxicity of oily DDT crystals may be derived entirely from the increased rate of penetration, across the cuticle, induced by oils.

Summary.

This paper presents the results of an investigation of certain processes taking place when flies of the species *Phormia* (*Protophormia*) *terraenovae* R.-D. are exposed to finely particulate deposits. Information is advanced concerning the precise sites of uptake of particles on the tarsi and pretarsi, the effects of "cleaning" reflex movements on the location and retention of particles and the influence of lipid solubility (a fundamental property of contact insecticides) on the adhesion of dry particles to the lipophilic surfaces of insect and leaf cuticle.

A technique for the controlled exposure of active blowflies of the species *P. terraenovae* to particulate deposits is described.

The rate of uptake of particles by walking flies is found to decrease rapidly during the first few seconds of exposure. Particles are taken up principally by the ventral tarsal spines and in smaller quantities by the pulvilli. The frontal membranes of the contact chemoreceptors also became contaminated but the intersegmental joint membranes are defended from particles by large spines that

become heavily contaminated. Certain tarsal segments collect particles more efficiently than others and it is deduced from the results obtained that "hairiness" and the pressure of segments against the substrate are important variables affecting the rate of contamination. Cleaning movements continually transfer particles in constant proportions to the basal parts of tarsal setae, to the microtrichia of the tarsal cuticle, to the head, wings and abdomen.

It is considered that this phenomenon must greatly facilitate the toxic action of an insecticidal deposit.

The majority of particles discarded by a contaminated fly is transferred from the tarsal setae to clean surfaces by a reversal of the pick-up process. These setae recover particles, by cleaning, from other parts of the body. A second discarding mechanism of less importance is provided by the cleaning reactions in which fine particles are gradually combed into larger aggregates that can be knocked off.

The rate of loss of particles from active adults of *Phormia* is relatively rapid until the quantity retained has fallen to about 20 μg . and is very slow below 10 μg . Particles at any given dosage are discarded at the same rate whether they have been acquired during continuous or intermittent exposures of flies to deposits.

Dry particles of a lipid-soluble substance have been found to adhere more strongly to the cuticle of adults of *Phormia* and to a leaf wax than do dry particles of a substance insoluble in lipids. The presence of an oil on a substrate hinders the uptake of particles to a marked degree.

Acknowledgements.

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THE ACTIVITY CYCLE OF DOMESTIC *AÊDES* (*STEGOMYIA*)
AEGYPTI (L.) (DIPT., CULICID.) IN SOUTHERN
 PROVINCE, TANGANYIKA.

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In the vast body of literature relating to the mosquito, *Aedes* (*Stegomyia*) *aegypti* (L.), there is surprisingly little on the periodicity of activity of the adult, despite the obvious importance of this aspect of its biology with regard to the transmission of parasitic organisms. For instance, Horsfall (1955), in a summary of its ecology extending to 29 pages, devotes less than a quarter of a page to periodicity of biting activity and, with one exception, the papers he quotes date from the first few years of the century. However, it is to be concluded from his summary that *A. aegypti* bites mainly in the daylight hours, and shows two peaks of biting activity, one "early in the morning" and the other "during the late afternoon", the latter the more pronounced. Information with regard to night feeding is less definite but Horsfall mentions that "older mosquitoes seem to extend the feeding period into the night".

Since the time of the authorities quoted by Horsfall, some more detailed information has become available as a result of catches of the continuous baited type as originally devised by Kerr (1933) and developed and standardised by Haddow (1954). Kerr himself records that *A. aegypti* was abundant in native habitations in Nigeria but unfortunately he made catches only outside, and the numbers which he records are only small. More recently, Teesdale (1955) has recorded the results of baited catches, covering the whole or a large part of the 24-hour period, in several environments near Mombasa, Kenya. He was working in an area in which the populations of *A. aegypti* were comparatively small and it was sometimes necessary to combine the results of several years to obtain numbers sufficient to define the curve of biting activity. In 1953, during the course of an investigation into a severe epidemic of a virus disease that affected the population of the Makonde plateau in the Newala district of southern Tanganyika in 1952-55 (Lumsden, 1955), an opportunity offered to study the periodicity of activity of *A. aegypti*, by means of the continuous baited catch, in a locality in which it was extremely abundant. Although the catch was of comparatively short duration, only 49 hours, 603 males and 2,594 females of *A. aegypti* were taken by two teams of four catchers; and from these large numbers it is possible to define the alterations of activity from hour to hour without recourse to the compounding of the data of several separate days, as is usually necessary. It is the purpose of the present paper to record the activity rhythms found, to compare them with Teesdale's findings and to discuss what factors might determine them.

The topography, climate, hydrography and vegetation of the locality studied, Newala district, Southern Province, Tanganyika, and the customs of its people, have already been described (Lumsden, 1955) so that a few basic facts will suffice here. The district consists mainly of a sandstone plateau lying at about 2,000 ft. (600 m.) above sea-level. The sandstone is highly permeable and the plateau surface is practically devoid of water, a circumstance which induces the inhabitants, mainly of the Makonde tribe, to store this. They collect water during rainstorms, or carry it up from the lower lands, and store it in spherical earthenware pots, holding up to 7 or 8 gallons, which they dig into the floors of their

huts. In a sample of 213 houses the average number of pots per house was 9.0, and of water-filled pots, 4.0, but sometimes as many as 20 water-containing pots could be found; characteristically, mosquito larvae abound in the pots. Also there are often many jars, or fragments of jars, scattered outside the huts.

The huts are generally square in plan, of wattle and daub, and thatched. The roof overhangs the wall enclosure by a yard or so, forming a narrow verandah. Windows are small or absent and only a narrow space is left between the walls and the roof. The hut interiors are practically dark even by day. The huts are divided up internally by partitions, and there is usually an open ceiling of grass stems laid horizontally. Beds are of woven grass on wooden frames. Pigeons, fowls and dogs are sheltered in the huts as well as men.

The Conditions under which the Catches were made.

The catches were made inside and on the verandah of a typical hut, which was the only one permanently occupied of a group of three, at Mpembe, about two miles north of Newala township. The hut was occupied by an old man and his wife who followed their normal routine during the course of the catch, frequently entering the hut in the daytime, and sleeping in it at night.

As sunset times were not available, the catches, as they were carried out, had to be related to East African Standard Time. The times of sunrise and sunset at Newala on 13th March 1953, calculated subsequently, were 0628 and 1839 hr. E.A.S.T., respectively. Therefore, in order to approximate as closely as possible to "catch time" (Lumsden, 1952), one hour has been subtracted from all timings on East African Standard Time for record in the present paper, e.g., sunset falls at 1739 hr. (fig. 1).

The native catchers were mainly local recruits who had their first experience of mosquito work in the few weeks preceding the catch, but each team was under the supervision of a more experienced man, an African Health Inspector or a Malaria Control Foreman. I myself visited the catch every few hours throughout its duration. There were six teams, of four catchers each, and two teams were in action at any one time, one inside the hut and one on the hut verandah. The teams were changed at regular intervals, at 0600, 1400 and 2200 hr. The darkness of the hut interior necessitated the use of hurricane lamps to see the mosquitos alighting on the bait, by day as well as by night.

The catch was first started at 1000 hr. on 12th March but had to be abandoned after about half an hour as all the catching tubes available had been filled. Mosquitos taken at that time have been excluded from the present discussion as the exact duration of catching was unknown. Glass tumblers, covered with calico, were then improvised as containers for the mosquitos and the catch was resumed at 1300 hr. Thenceforward, the mosquitos were caught singly in glass tubes, which were then plugged with cotton-wool in the usual manner, and the mosquitos were later transferred to the tumblers, so releasing the tubes to be used again.

The 12th March was cool with cloud and slight wind and rain; it was followed by a cold, very dark night with thick cloud. The 13th was a calm, sunny day and was followed by a cold, calm night, with some rain. The 14th was calm and sunny.

A knockdown catch with a very heavy dose of pyrethrum spray, made after the end of the 49th hour of the continuous baited catch, from 1415-1430 hr. on the 14th March, was intended to assess the mosquito populations remaining in the hut after 49 hours of continuous catching.

The Results of the Catches.

Only two species of mosquito were taken, *A. aegypti* and *Culex* (*Culex*) *pipiens fatigans* Wied.

TABLE I.

The numbers of males and females of *Aedes aegypti* taken by two teams of native mosquito catchers. inside a hut and on its verandah, at Mpenbe, near Newala, Tanganyika; 12th-14th March 1953.

		Hour beginning:																							Totals		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22		23	
♂♂	Hut interior	12 Mar.	—	—	—	—	—	—	—	—	—	—	—	—	—	5	11	22	1	2	0	2	5	2	0	1	51
		13 Mar.	1	2	4	8	0	1	3	26	20	4	3	2	10	2	6	5	4	3	0	1	3	4	1	2	115
		14 Mar.	0	0	7	6	1	1	8	11	12	12	6	9	6	4	—	—	—	—	—	—	—	—	—	—	83
		Totals	1	2	11	14	1	2	11	37	32	16	9	11	16	11	17	27	5	5	0	3	8	6	1	3	249
		M _G	0.4	0.7	5.0	6.9	0.4	1.0	5.0	17	15	7.0	4.3	4.5	7.8	3.5	8.2	11	2.2	2.5	0	1.4	3.9	2.9	0.4	1.4	—
	Hut verandah	12 Mar.	—	—	—	—	—	—	—	—	—	—	—	—	—	12	25	24	4	2	0	0	0	0	0	0	67
		13 Mar.	2	1	0	0	3	4	10	6	23	21	22	9	18	23	13	23	10	12	0	0	0	0	1	0	201
		14 Mar.	2	0	0	1	1	1	6	10	21	26	7	2	4	5	—	—	—	—	—	—	—	—	—	—	86
		Totals	4	1	0	1	4	5	16	16	44	47	29	11	22	40	38	47	14	14	0	0	0	0	1	0	354
		M _G	2.0	0.4	0	0.4	1.8	2.2	7.8	7.8	22	23	13	4.5	8.8	11	18	24	6.4	5.2	0	0	0	0	0.4	0	—
	Hut interior	12 Mar.	—	—	—	—	—	—	—	—	—	—	—	—	—	64	60	66	48	36	11	17	32	50	4	16	404
		13 Mar.	9	8	30	71	28	32	40	19	106	40	54	27	56	62	139	80	68	31	34	41	53	57	11	21	1117
		14 Mar.	23	10	40	31	22	24	28	24	53	48	46	29	50	24	—	—	—	—	—	—	—	—	—	—	452
		Totals	32	18	70	102	50	56	68	43	159	88	100	56	106	150	199	146	116	67	45	58	85	107	15	37	1973
		M _G	14	8.9	34	47	25	28	33	21	75	44	50	28	53	46	92	73	57	34	19	27	41	53	6.8	18	—
	Hut verandah	12 Mar.	—	2	15	—	—	—	—	—	—	—	—	—	—	15	12	9	3	9	2	1	4	0	2	4	61
		13 Mar.	2	2	15	0	12	23	3	31	29	22	8	4	24	41	14	16	13	41	20	8	5	3	5	16	357
		14 Mar.	6	12	5	4	19	27	7	16	23	15	22	17	14	16	—	—	—	—	—	—	—	—	—	—	203
		Totals	8	14	20	4	31	50	10	47	52	37	30	21	38	72	26	25	16	50	22	9	9	3	7	20	621
		M _G	3.6	5.2	8.8	1.2	15	25	4.6	22	26	18	13	8.5	18	21	13	12	6.5	19	6.9	3.2	4.5	1.0	3.3	8.2	—

A. aegypti was extremely abundant and both males and females occurred on the bait. A considerable proportion of the population consisted of the pale form, in which, in its fullest expression, the normally black mesonotal scales were pale golden yellow. Intergradations between the normal and the fully pale form occurred. Paleness appeared to be expressed more commonly in the female than in the male; of 12 females pinned at random, eight were fully pale, two intermediate and two normal, while of 40 males also chosen at random, only one was fully pale, 11 were intermediate and 28 were normal.

C. p. fatigans was, in the particular hut chosen for the catch, rather rare; only 10 males and 21 females were taken during the whole catch. It was, nevertheless, generally common as a larva in pots and was about as common as *A. aegypti* in a larger sample; 519 knockdown catches in huts in Newala and Masasi districts, most of them on the Makonde plateau, yielded 746 examples of *A. aegypti* and 771 of *C. p. fatigans*.

The knockdown catch made at the end of the continuous baited catch yielded 106 males and 107 females of *A. aegypti* and 20 males and 28 females of *C. p. fatigans*. Although in this catch *A. aegypti* still outnumbered *C. p. fatigans*, the proportion of *C. p. fatigans* was very much higher than in the continuous baited catch; it appears from the results of precipitin tests (Lumsden, 1955) that *A. aegypti* in Newala feeds exclusively on man while *C. p. fatigans* bites fowls also.

C. p. fatigans need not be further referred to but it is of interest to record the total numbers taken in the various sections of the catch:—

	♂♂	♀♀
Hut interior	7	19
Hut verandah	3	2
By night	7	15
By day	3	6

The periodicity of occurrence of *A. aegypti* on the bait is shown numerically in Table I and graphically in figs. 1 and 2. Only a brief description is necessary.

The main activity of females of *A. aegypti* is diurnal but the night activity is by no means insignificant. Apportioning the numbers caught, during the hours in which sunrise and sunset fell, to night or day in proportion to the fraction of these hours before or after the sun's passing the horizon, and rejecting the 49th hour (beginning 1300 hr. on 14th March), the following totals for females caught are obtained:—

	Hut interior	Hut verandah
By day	1280 (66%)	416 (69%)
By night	669 (34%)	189 (31%)

Thus, both in the hut and on its verandah, about two-thirds of the 24-hours' biting activity takes place by day, about one-third by night.

Inside the hut, females of *A. aegypti* show four well-marked waves of biting activity in each 24-hour period (fig. 1). Each wave appears to be highly consistent, occurring quite clearly twice during the two days of the catch. Two of the main waves occur by day and two by night. Day-time peaks of biting activity fall in the hours 0800–0900 and 1400–1500, night-time peaks in the hours 2100–2200 and 0300–0400. There are signs of other, subsidiary, waves of biting

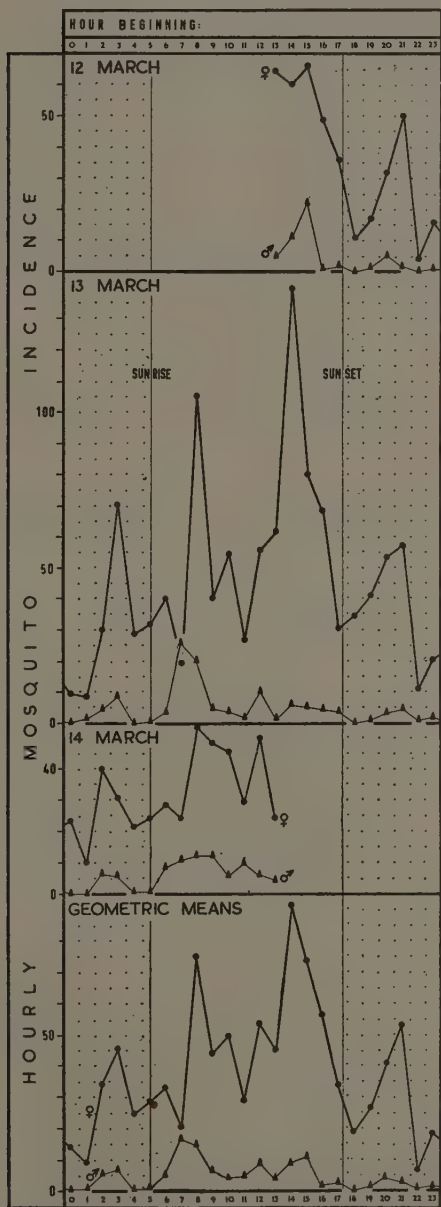


Fig. 1.—The incidence of males and females of *Aedes aegypti* on a team of four native catchers at Mpembe, Newala district, Tanganyika; 12th–14th March 1953. Hut interior.

activity. For instance, on both nights there was a smaller wave between 2200 and 0100 hr., with a peak in the hour 2300-midnight, or midnight-0100, and another between the late night and the first day-time main activity wave with a peak during the hour 0600-0700. The nocturnal activity peaks are of about the same amplitude, giving peak geometric-mean biting rates of about 50 mosquitos per hour. The diurnal activity waves both exceed this level, and the afternoon peak exceeds the morning one; the peak geometric-mean biting rate is about 75 mosquitos per hour in the morning, about 90 in the afternoon. For convenience in description and comparison, the main waves of activity will be called the first, second, third and fourth main waves, counting beginning with the post-midnight nocturnal wave between 0200 and 0400 hr. Thus the nocturnal main activity waves are the first and fourth, the diurnal ones the second and third.

Outside the hut also, activity occurs in a series of waves but the waves are not all coincident with those occurring inside the hut (fig. 2). There appear to

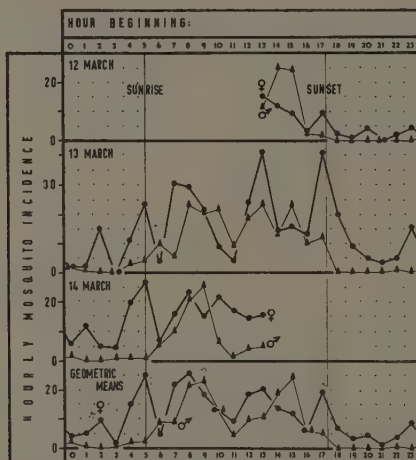


Fig. 2.—The incidence of males and females of *Aedes aegypti* on a team of four native catchers at Mpembe, Newala district, Tanganyika; 12th-14th March 1953. Hut verandah.

be six waves of activity outside the hut and the waves tend, as in the case of the waves of activity inside the hut, to be repeated consistently from day to day. They will be referred to as the first to sixth activity waves for comparison with the activity rhythm inside the hut. The first wave reaches a peak in the hours 0100-0200 or 0200-0300. The trough between it and the second wave corresponds with the first main wave of activity inside the hut. The second wave falls about sunrise, *i.e.*, approximately coincidently with the subsidiary wave inside the hut at that time. The third wave reaches a peak at about 0800 hr., coincidently with the second main wave inside the hut. The fourth wave precedes slightly the third wave inside the hut. The fifth wave reaches a maximum just before sunset, nearly coincidently with the trough between the third and fourth waves inside the hut. The sixth wave shows a peak in the hour 2300-midnight, at the same time, approximately, as one of the subsidiary waves inside the hut.

The incidence of males of *A. aegypti* on the bait was mainly diurnal. The

day and night totals, calculated in the same way as was done for the females (see above) were:—

	Hut interior	Hut verandah
By day	192 (78%)	330 (95%)
By night	53 (22%)	19 (5%)

Activity is more diurnal than in the case of the females, particularly out-of-doors, where the numbers captured by night were very small. Inside the hut, the periodicity of incidence of the males corresponds closely to that of the females but is at a lower level. There are four main waves of activity, the second the most marked. The day-time rhythm outside the hut also corresponds closely in time to that of females inside the hut; the waves of activity are very well marked, being composed of larger numbers than occur inside the hut. The virtual absence of males outside at night is striking.

Discussion.

The relation of the biting habits of *A. aegypti* in Newala district to the transmission of virus infections has already been considered elsewhere (Lumsden, 1955) and will be excluded from the present discussion. It is, however, of interest to estimate the amount of blood removed daily by *A. aegypti* from an inhabitant of such a highly infested hut. Taking the average blood-meal of a female of *A. aegypti* as 2.5 mg. (Horsfall, 1955), the specific gravity of blood as 1.057 (Stitt, 1923) and allowing for 484 bites per inhabitant per 24 hours, the amount required is 1.14 ml. per day. The quantity is remarkably small; it may be compared with the daily consumption of a single hookworm, given as 0.84 ml. by Whitby & Britton (1950).

Before discussing the activity rhythms found, it is necessary to consider two points. The first is whether the change over of teams could have any marked effect on the efficiency of catching and so on the numbers of mosquitos caught. Such an effect is particularly possible in the present catch as it was made with relatively inexperienced men. Neglecting variations in attractiveness to mosquitos, and in skill, on the part of individual catchers, which in a team of four people may be expected largely to equalise each other, it might be expected that a fresh team would be more efficient than a tired one and that a spurious rise might take place at the time of taking over of the fresh team. No such effect appears, indeed in most cases the first hour of work of each team coincides with low numbers. It is considered that effects of this sort may also be safely ignored; in any case the hour-to-hour variations in the numbers of mosquitos caught are large, e.g., in the case of females within the hut from a minimum of 4 to a maximum of 139 mosquitos per hour, a range of from 0.12 to 4.2 times the geometric-mean number (33) caught per hour over the whole catch. In an adequately supervised catch it does not seem likely that variations in efficiency could account for more than a small fraction of this range. The second point concerns the influence of the inhabitants of the hut on the numbers of mosquitos taken by the teams. It is clear that they formed another focus of variable attraction which may have drawn off mosquitos which might otherwise have occurred in the catch. However, only two in number, they were probably less attractive than the teams, especially by day when they were awake and active; at night, asleep, they may have been of more importance and drawn off a larger proportion of the mosquitos coming to bite, possibly thus contributing to a reduction of the night-time as compared with the day-time peaks.

Kerr (1933) gives no information about the behaviour of *A. aegypti* in houses in Nigeria beyond noting that it was abundant and active there. In a series of his standard night catches out-of-doors, he took the occurrence of 5 specimens in the 90 minutes succeeding sunset, and one in the next 90 minutes, together with the general rarity of the species in night catches, to indicate that it was mainly crepuscular in its time of activity.

Teesdale (1955) in a long series of baited catches made out-of-doors in Kenya, from dawn to dusk by hourly intervals, found the main activity wave to begin in the hour 1600-1700 and to reach a peak soon after sunset. He also found some indication of another wave of activity extending from 1000 to 1300 hr. However, although over 6,000 mosquitos were taken in the nearly three years of catching, the intensity of biting was never very high, never exceeding an average of 5 mosquitos per hour per team of 3-9 catchers, even in the crepuscular period. Teesdale (1955) also made series, each of fifteen 24-hour baited catches in five other environments, *viz.*, at three levels in a mango tree, and inside and outside a house. Biting intensity was never very high, only 352 mosquitos being taken altogether in the five series, involving 1,800 hours' catching, and the highest arithmetic mean number of mosquitos attacking per hour being only 1.7, but the rhythms of activity are clear in three of the five environments which he studied. Considering his hourly figures (as I consider that compounding of the data of separate hours should be avoided as liable artificially to displace activity peaks in time), the defined rhythms (from 0600 to 1800 hr. being considered day, and the other half of the 24 hours, night) are as follows:—

Thirty seven mosquitos were taken on the ground under a mango tree, 26 (70%) by day and 11 (30%) by night. There was a period of activity, but at a very low level, from 0600 to 1000 hr. No mosquitos were taken in the succeeding two hours and another wave of activity began in the hour 1300-1400, reached a peak in the hour 1700-1800, just before sunset, and ended in the hour 2000-2100. No mosquitos were taken between 2100 and 0600 hr.

One hundred and seventy nine mosquitos were taken in a village outside a house, 133 (74%) by day and 46 (26%) by night. There were two main waves of activity, one in the morning between 0500 and 1200 hr. and one in the afternoon in hour 1700, just before sunset.

One hundred and eighteen mosquitos were taken in the house, 95 (81%) by day and 23 (19%) by night. There were two main waves of activity, one in the morning between 0900 and 1200 hr. and one in the evening in hour 1800-1900, just after sunset.

There appears to be no fundamental difference between any of the rhythms found by Teesdale for *A. aegypti* in the several environments. All consist essentially of two main waves, one typically of slow rise and fall and long duration, from about dawn to about noon, and another, shorter and more abrupt, falling apparently sometimes just before, sometimes just after, sunset.

The morning wave shown in Teesdale's work appears roughly to coincide with the second main wave of activity inside the hut and the third wave of activity on the hut verandah. The wave nearly related to sunset appears to correspond to the wave at about the same time on the hut verandah, which was, however, in the present work, not represented inside the hut. It does appear from a survey of all the work, both Teesdale's and the present study, that there are seven times in the 24 hours at which activity waves of *A. aegypti* may occur and that the different curves are all modifications of the one fundamental system; but it should be remembered that some of the activity waves separated here may really represent the same outburst of activity manifesting itself at slightly different times in different places because of some unknown factor. These seven times may be considered in greater detail:

(a) The first wave, with a peak in the hours 0200-0300 or 0300-0400: the

first main wave of the hut-interior curve; the first wave of the hut-verandah curve; not represented on any of Teesdale's curves.

(b) The second wave, with a peak at, or soon after, dawn: represented in the hut-interior curve by a subsidiary wave; the second wave in the hut-verandah curve; faintly indicated in Teesdale's figures, for the ground below the mango tree, and inside and outside the house.

(c) The third wave, with a peak in the hour 0800-0900, or soon after: the second main wave in the hut-interior curve; the third wave in the hut-verandah curve; apparently slightly later in Teesdale's figures in the open, and inside and outside the house, the peak falling in the hours 0900-1000 or 1000-1100.

(d) The fourth wave, with a peak about 1400 hr.: the third main wave in the hut-interior curve; the fourth wave in the hut-verandah curve; faintly indicated in Teesdale's figures for inside and outside the house.

(e) The fifth wave, with a peak nearly at sunset: not represented in the hut-interior curve; the fifth wave in the hut-verandah curve; the second wave of Teesdale's figures for the open, the ground below a mango tree, and inside and outside the house.

(f) The sixth wave, with a peak in the hour 2100-2200: the fourth main wave in the hut-interior curve; not represented in any other curve.

(g) The seventh wave, with a peak about midnight: indicated as a subsidiary wave in the hut-interior curve; the sixth wave in the hut-verandah curve; indicated in Teesdale's figures for outside the house.

In the present work, night-time biting activity of *A. aegypti* composed 31-34 per cent. of the total; it was generally a little less than this (19-30%) in Teesdale's experience. But Teesdale quotes some results, due to Mattingly, which appear to indicate that the mosquito in huts in West Africa is largely nocturnal; 72 per cent. of a total of 57 taken biting in eight 24-hour catches occurred between 2100 and 0900 hr.; the corresponding figure for the present work is only 39 per cent.

The significance of the occurrence of males on the bait is uncertain. Horsfall (1955) says of *A. aegypti* "even males may become obnoxious as they dine on perspiration" and Bates (1949) quotes a statement that the males may take up positions on a person sitting quietly and wait for an opportunity to pounce on a female coming to feed. The rhythm of occurrence of males on the bait in the present catch has been remarked to resemble closely that of females inside the hut. It seems possible that a causal relationship is concerned here. Horsfall (1955) records that when females of *A. aegypti* vibrate their wings, males flock towards them, and Teesdale (1955) has shown by means of window traps that males of *A. aegypti* tend to leave a hut during two main periods daily, from 0600 to 0900 hr. and from 1500 to 1900 hr., and to re-enter in the middle of the day from 0800 to 1500 hr.

The mechanisms by which such periodicities of activity are determined are as yet not understood. Harker (1954, 1955) has shown that true physiological rhythms occur in the cockroach, *Periplaneta americana* (L.), and are controlled by the ocelli and the suboesophageal ganglia. Bates (1949), working in Albania, found *Anopheles superpictus* Grassi to behave in this manner but not *Aedes aegypti*. It is not known if East African populations of *A. aegypti* differ in this respect from the strain studied by Bates.

The microclimate of hut interiors has been studied by Hadow (1942) and Lumsden (1951). As far as temperature and humidity are concerned, conditions inside a hut tend to be more equable than in the open. It is difficult to see any relationship likely to be a causal one between the temperature and humidity variations and the variations in biting activity on the part of the mosquitos, except perhaps that activity in the midday period might be depressed by high temperature or low humidity or a combination of these two factors. Nor do

changes in light intensity seem likely to be concerned; inside the hut the waves of activity occur at times conspicuously unrelated to either dawn or dusk when light intensity changes might be expected to be most effective.

Genetically determined differences in behaviour between populations of *A. aegypti* in different areas, as have been shown by Gillett (1955, 1956), might be important, and even in the same environment the population might consist of more than one group differing in behaviour. In Newala, the population was made up of both apparently normally coloured and pale forms. Unfortunately, detailed notes were not kept of the hourly incidence of the two forms, but the pale form was taken biting both by night and by day.

Marchoux, Salimbeni & Simond (1903) and Marchoux & Simond (1906) have stated that *A. aegypti* in nature ceases to bite man by day after the first 6 to 8 days of adult life. The statement is based on their finding a considerable number of specimens taken biting by day in a uniformly unworn condition, and on experiments involving at their outsets less than 42 mosquitos. The results presented, though suggestive, cannot be considered more than that by modern standards. Various other authors have investigated the matter, or repeated the statement, and their opinions may be considered.

Macfie (1915) studied in detail the behaviour of individual females of *A. aegypti* confined in glass cylinders, offering them blood-meals usually twice each day, between 1300 and 1400 hr., and between 2000 and 2100 hr. He found that "mosquitoes of all ages might feed at any hour" but that the time of the blood-meal was related to the previous oviposition; in 35 of 54 instances the blood-meal was accepted at the first offering after the completion of oviposition. He found that "over 50 per cent of the eggs laid were deposited before 8 a.m.", i.e., between 9 p.m. (2100 hr.) the previous night and that time, the remainder mainly in the late afternoon or evening.

Gordon & Young (1921) kept females of *A. aegypti* in captivity for more than 14 days, feeding them on sugar solutions and allowing them two blood-meals. They then marked them by amputating their hind legs through the tibiae, released them in a building, and recorded the numbers which came to bite during one day-time and one night-time hour during several subsequent days, comparing the results with similar observations on unmarked mosquitos. In three experiments, 21 marked mosquitos were taken biting, 15 by day and 6 by night; 23 unmarked mosquitos occurred at the same times, 13 by day and 10 by night.

Flu (1928) estimated rates of infection with the larvae of *Wuchereria bancrofti* in *A. aegypti* and *Culex p. fatigans* in dwelling houses in Surinam. Of 215 females of *A. aegypti*, 8.3 per cent. were infected, and of 638 of *C. p. fatigans*, 27 per cent. As both species were about equally susceptible in the laboratory, he ascribed the difference in infection rates to differences in the times of activity of the two species, stating that *A. aegypti* "during the first few days of their life only fly by day, and after the third day of life bite only at night", while *C. p. fatigans* "bites when it is dark". As microfilariae occurred in the peripheral blood of the infecting host only by night, he considered that *C. p. fatigans* was exposed to infection from its earliest blood-meals, *A. aegypti* only several days after eclosion from the pupa. However, he does not give the evidence on which he bases this conception. It is not necessary to postulate such an alteration in habit to account for the difference in the infection rates; if the infective forms occur in the peripheral blood of the infecting host only by night, and other factors are equal, then a lower infection rate would be expected in a mosquito largely diurnal in its biting habits as compared with another largely nocturnal.

Also Cardamatis (1929), working in Greece, states that *A. aegypti* bites at first in daylight, later at night, but does not give any evidence to support the statement.

Neither the experiments of Macfie nor those of Gordon & Young support the statement made by Marchoux and his co-workers, and other writers who have made the same statement, including Horsfall (1955), have not adduced evidence to prove it. Nevertheless, it must be admitted that the experiments of Macfie and of Gordon & Young were all concerned with only small numbers under highly unnatural conditions and in the face of some recent observations the statement cannot be summarily dismissed.

Davies (1955) has found in *Simulium ornatum* Mg., in which the character of the fat-body changes with age, that the population biting in the late evening is composed of older flies than that which bites by day.

Lumsden (1952) pointed out that, if different age-groups bit at different times, then the form of the biting-activity curve might be expected to change with variations in the constitution of the population. He suggested that, in the case of nocturnally active mosquitos, the first wave of activity after sunset might be mainly composed of older mosquitos, while those attacking later in the night might be mainly newly-hatched females whose advent had been delayed by some other activity, such as mating. Senior White (1953) investigated the evening activity of *Anopheles aquasalis* Curry, *A. albitarsis* Lynch-Arrib. and *A. neomaculipalpus* Curry in Trinidad, British West Indies. He dissected a large proportion of his catches and he records the ovarian stages (Christophers, Sinton & Covell, 1939) of the mosquitos coming to bite, by ten-minute periods from 20 minutes before, to 2 hours after, sunset. It is clear from his work that age changes, if they occur, are not clear-cut, as he found that 18.3 per cent. of the 3,146 females of *A. aquasalis* dissected were in ovarian stage 1, and therefore must be supposed to be biting for the first time. He did not find a rise in the proportion of females feeding for the first time after the initial crepuscular wave of activity, rather the reverse, but unfortunately his catch ended two hours after sunset and data on the age of the mosquitos biting later in the night are not available; they may be expected to outnumber those biting up to 2000 hr. (Senior White, 1951). Considering his results in another way, it can be said that a very high proportion of the population of *A. aquasalis* biting in the first two hours after sunset is biting for the second or a subsequent time; percentages biting with the ovaries in stages 2 and 3 vary between 75 and 90. In the case of *A. albitarsis*, this state of affairs is even more marked, as in these two hours, 329 out of 342 dissected, or 96 per cent., showed ovaries in stages 2 and 3. In *A. neomaculipalpus* the proportion was smaller but was still 68 per cent. The significance of these high percentages of mosquitos taking their second or subsequent meal just after sunset is uncertain in the lack of information on the representation of these ovarian stages in the general population. However, Senior White (1951) has adduced evidence to show that only about 5 per cent. of freshly fed *A. aquasalis* survive to mature their ovaries, i.e., that a reasonable estimate of the mortality in one gonotrophic cycle is 95 per cent. If this is so, then it would be expected that at any given moment the mosquitos ready to bite would comprise approximately 95 per cent. individuals feeding for the first time and 5 per cent. those feeding for the second time, those feeding for the third and subsequent occasions being an insignificant proportion. Thus, if the population biting in the first two hours is largely composed of mosquitos biting for the second or subsequent time, as Senior White has shown to be the case, it would seem that the mosquitos biting later in the night must be mainly feeding for the first time. However, it should be noted that even if all were so, on Senior White's figures (1951, text table on p. 467; 1953, Table I), the proportions of mosquitos feeding for the first and subsequent times should be, respectively, about 65 and 35 per cent., indicating a mortality very much lower than the proportion of 95 and 5 per cent., respectively, derived from his data for the resting population (Senior White, 1951).

Gillies (1957) has recorded some data relating to this matter for *Anopheles*

gambiae Giles biting in huts in East Africa. In his catches (which were divided into three periods from sunset to sunrise) he was able to separate a group which was composed of at least 91 per cent. females feeding for the first time ("pre-gravid" females) and another composed of females taking later meals ("gravid" females), and to calculate the geometric-mean numbers attacking for the two groups separately. His results were as follows:

		Time intervals		
		1830-2200 hr.	2200-0200 hr.	0200-0530 hr.
Pre-gravid females	..	6.3	13.1	11.8
Gravid females	..	11.6	39.3	26.9

It is clear from these results that different age-groups do not attack as distinct entities, at least over time intervals of this length, and that the periodicity of the females feeding for the first time resembles broadly that of the older females. However, the variations in the geometric means are proportionately much wider in the case of the gravid than in that of the pre-gravid females and it is evident that the former group would contribute much more than the latter to the contour of a curve in which the two groups were unseparated. With respect to mating, which was advanced (Lumsden, 1952) as an activity which might possibly delay the advent of newly hatched females until later in the night, Gillies' two age-groups do not differ, as about three-quarters of the pre-gravid group are unfertilised (Gillies, 1957), indicating that they do not delay their attack until mating is accomplished. It seems more likely in this case that oviposition, which Muirhead-Thomson (1940) has shown, for *Anopheles minimus* Theo., to take place mostly in the first third of the night, delays the advent of the older females to bite. Gillies also adduces evidence that the age composition of the gravid group is not uniform through the night; the malaria sporozoite rate tended to fall as the night advanced, and the rate in the terminal third of the night was significantly lower than the all-night rate, indicating that the mosquitos biting at that time were on an average younger than the rest. On the other hand, Gillett (personal communication), working with a swamp mosquito and taking infection with mites to indicate a "young" mosquito, has found little difference in the proportional representation of that age-group through the night.

It is concluded that studies of mosquitos directed to their ages at the time of biting, to their intrinsic rhythms, and to the periodicity of aspects of their behaviour other than biting, as, for example, oviposition, are most likely to contribute largely to an understanding of the mechanism of determination of the biting cycles.

Summary.

The population of the Makonde plateau, Newala district, Tanganyika, underwent a severe epidemic of a virus disease in 1952-55, and during an investigation of the epidemic the opportunity was taken to define the biting activity cycle of *Aedes (Stegomyia) aegypti* (L.), a mosquito that was extremely common in native huts on the plateau.

Continuous baited catches, lasting for 49 hours, were made by teams of four native catchers, inside a hut and on its verandah. The intensity of attack is expressed as the geometric-mean numbers of *A. aegypti* alighting on such a team per hour. The catches are recorded on a 24-hour system in which sunset is near to 1800 hr.

The total numbers of females of *A. aegypti* taken were 1,973 inside the hut and 621 on its verandah. The main activity of the females of *A. aegypti* was diurnal, 66 per cent. of the catch inside the hut, and 69 per cent. of the catch on the verandah, being taken between sunrise and sunset. Inside the hut there were four main waves of biting activity, consistent on the two days of the catch. Two fell by day, with peaks in the hours 0800–0900 and 1400–1500, and two by night, with peaks in the hours 2100–2200 and 0300–0400. There were signs of other, subsidiary waves. Outside the hut there appeared to be six waves of biting activity; four of these fell at the same time, or close to, main or subsidiary waves inside the hut, but two, notably the wave about sunset, were not so related.

Males of *A. aegypti* also occurred on the bait, 249 being taken inside the hut and 354 outside it. There was less nocturnal activity than in the case of the females; only 22 per cent. of the total catch inside the hut and 5 per cent. of that outside it were taken by night. The periodicity of incidence of males corresponded closely with that of the females both inside and outside the hut, except that males were virtually absent outside by night.

The rhythms of activity found are compared with the results of other workers, and it is concluded that studies of mosquitos directed towards their ages at the time of biting and to periodicity of aspects of their behaviour other than biting are most likely to contribute to an understanding of the mechanism of determination of biting cycles.

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STUDIES ON THE RESPONSES OF THE FEMALE *AÈDES* MOSQUITO.

PART VIII.*—THE ATTRACTIVENESS OF BEEF BLOOD TO *AÈDES AEGYPTI* (L.).

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R.L.

The first attempts to demonstrate the attractiveness to mosquitos of mammalian blood proved unsuccessful. Adults of *Culex pipiens fatigans* Wied. and *Aedes* (*Stegomyia*) *scutellaris* (Wlk.) were observed to ignore a small pool of blood exposed in a room (Howlett, 1910). *Aedes sollicitans* (Wlk.) and *A. cantator* (Coq.) did not react to the exposure of fresh human blood or beef blood (Rudolfs, 1922). Once clotting was prevented, blood could be exposed over a large surface and could freely emit its volatile factors. Thus defibrinated pig blood exposed on filter paper placed on an internally-warmed "artificial arm" proved generally attractive to *Anopheles maculipennis atroparvus* van Thiel, although the preference over water alone or red-coloured water was slight and erratic (Reuter, 1936). In experiments with this species in the open air, large boxes containing a source of heat, and in which cloths were suspended, attracted approximately eight times as many adult females when the cloths were soaked in defibrinated pig blood as when they were soaked in water (van Thiel & Weurman, 1947).

The addition of fresh blood to fly-papers was found to increase the catch of adult *Culex pipiens* L. and *Anopheles maculipennis* Mg. by ten times (Schaefferberg & Kupka, 1951), but individual blood proteins, lipoids or haematin were inactive. These authors report results obtained with a "Blutduftstoff" prepared from blood (the species was unfortunately not stated) by Dr. O. Ballaus in the form of a colourless aqueous solution, whose sweet aromatic taste somewhat resembled that of blood. They tested 100- to 2,000-fold dilutions of this solution (now tasteless to man) and found them significantly attractive to adult *Culex pipiens* as well as to *Stomoxys calcitrans* (L.), but not to *Musca domestica* L. When exposed in dishes of salt-cellar size, certain dilutions of "Blutduftstoffe" were five times as attractive as water, and the attractiveness increased with air temperature and with concentration. Even when covered with an animal membrane or a filter paper, these solutions were still attractive and induced the mosquitos to feed through the membrane.

When olfactometer technique was applied to *Anopheles m. atroparvus*, it was found that the vapour obtained by passing air through citrated rabbit blood was more than twice as attractive as that from physiological saline (Laarman, 1955); beef blood, however, gave inconsistent results with this species. Laarman concluded that blood certainly contains odorous substances; for when the vapour from a confined rabbit was tested in the olfactometer it was very highly attractive even when carbon dioxide had been removed and the factor of water-vapour equilibrated with the control air-stream.

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The purpose of the present investigation, which was performed before the results of Laarman were published, was to ascertain whether mammalian blood was attractive to females of *Aedes aegypti* (L.), and if so, whether the attractiveness was due to its content of carbon dioxide.

Material and Methods.

The adult female mosquitoes used in this investigation were obtained from a stock of *Aedes aegypti* reared in a greenhouse according to the method described by Brown, Sarkaria & Thompson (1951). Pupae were introduced continuously into a large cage, $10 \times 4\frac{1}{2} \times 8$ ft., which thus contained 1,000–4,000 mosquitoes at any one time. These were fed on strings of water-soaked raisins, and several times a week were allowed to take a blood-meal from the back of a rabbit.

To minimise variations in behaviour due to age and nutritional status, some experiments were performed with even-aged groups of mosquitoes in a smaller cage, $35 \times 35 \times 24$ in. high, with a glass front. These groups, numbering 300 to 400 females, were obtained by emergence from pupae over a period of three days. They were never offered a blood-meal, but were fed either on raisins or on a glucose solution in a cellucotton pad. All experiments in either type of cage were performed in the greenhouse at 80–85°F. and 50–70 per cent. R.H.

The blood used for these studies was obtained every week from freshly killed beef cattle in a packing house. It was collected in sterile 1-litre flasks containing 50 mg. of heparin in 16 cc. of 0.9 per cent. NaCl. The flasks were then capped with wax paper and held in a refrigerator until the blood was used.

In the most simple experimental method, the material to be tested (5 cc. of heparinised blood, or the material derived from this) was added to a pad of three thicknesses of 9-cm. filter paper, which rested on the flat surface of an inverted 10-cm. petri dish. It was compared with a control material similarly exposed, the two dishes being placed 16 in. apart on the bottom of a white enamel tray resting on a stool inside the large cage. In later experiments the petri dishes were set in two holes in a black box on a trolley, which could be rolled into the small cage when required.

Further experiments on the vapour emanating from blood were performed in the large cage with an olfactometer, that described by Brown, Sarkaria & Thompson (1951) being used initially. The emission ports consisted of two conical Buchner funnels, 3.5 in. in diameter and covered with black net, set upright 16 in. apart in a black board suspended at a height of 4 ft. from the floor of the large cage. Provision was made for removal of the air immediately after emission from each port. The blood and control substances were exposed as a surface film on

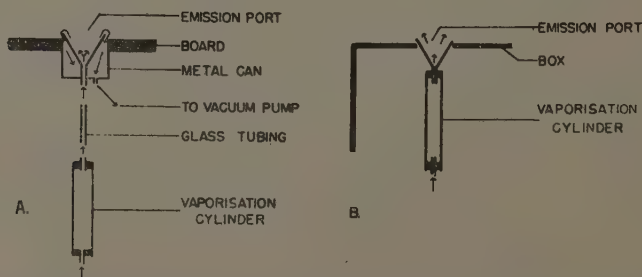


Fig. 1.—Arrangement for supplying vapour to the ports of the olfactometer. A, vaporisation cylinder set in a water-bath and connected by glass tubing; B, vaporisation cylinder placed immediately below the ports.

glass fragments in 2 x 10-in. glass vaporisation cylinders, immersed in a constant-temperature water-bath, and connected to the emission ports by a 6-ft. length of glass tubing (fig. 1A). Water-washed air from a cylinder was passed through each vaporisation cylinder to each port at 1.5 litres per minute.

This apparatus was later modified so that the vaporisation cylinders were located immediately below the ports (fig. 1B), which consisted of glass funnels mounted in holes in the bottom of an inverted black box. The apparatus was placed on a piano stool so that the position of the funnels could be reversed without removing them. The test and control materials were volatilised from a fluted filter-paper cartridge, glass fragments having proved unsatisfactory in this modification. Air was supplied to each port at 500 cc. per minute, and was not removed after emission.

The number of approaches made by adult mosquitos to within 0.5 in. of the top of the ports or petri dishes was counted by two observers for a set period of time, usually one or two minutes. The observers changed position after every count and the positions of the ports or petri dishes were interchanged after every second count. The mosquitos counted were almost exclusively females, since males seldom came to the ports. A complete experiment involved eight paired counts, and usually three such experiments were performed to investigate each point of comparison.

The attractiveness of the test material, as compared with a control, was expressed as an "Attractiveness Ratio", it being the number of mosquitos attracted to the test material divided by the number attracted to the control. The significance of differences between test and control materials was evaluated by Student's test. The value of t was calculated for the eight counts of each separate experiment, and for all the counts in the entire set of experiments. The 5 per cent. level ($p = 0.05$) was taken as the criterion of significant difference.

TABLE I.

Attractiveness of whole blood compared with water and washed corpuscles, and of plasma compared with water.

Expt. No.	Number of mosquitos		Ratio	t
	Blood	Water		
1	368	76	4.84	11.27
2	138	52	2.65	5.43
3	297	93	3.19	6.85
Total	803	221	3.63	9.17
	Blood	Corpuscles		
4	163	44	3.70	4.21
5	931	422	2.21	11.38
6	248	107	2.32	6.68
Total	1342	573	2.34	4.29
	Plasma	Water		
7	271	47	5.77	2.37
8	144	28	5.14	1.58
9	205	57	3.60	2.19
Total	620	132	4.70	3.64

Results.

The initial experiments were designed to ascertain the attractiveness of whole beef blood to *Aedes aegypti*, and to determine whether the attraction lay in the corpuscles or the plasma. After being heparinised, 5 cc. of whole blood were added to a pad of filter paper in a petri dish and compared for attractiveness with a similar amount of distilled water similarly exposed in the large cage.

The numbers of mosquitos coming to each (Table I) showed that whole beef blood was three to four times more attractive than distilled water. Then the whole blood was compared with a suspension of corpuscles, obtained by centrifuging 5 cc. of blood, washing seven times in 0.9 per cent. NaCl, and finally suspending them in this isotonic saline. The results obtained indicated that whole blood was two to three times more attractive than the suspension of corpuscles, which closely matched it in colour. The attractiveness of whole blood, therefore, was due to some factor in the plasma. When the plasma obtained from 5 cc. of blood was compared with water, it proved to be 4 to 5 times more attractive. All the differences observed were of high statistical significance.

The attractiveness of the vapour from blood was then investigated in the olfactometer, by comparing it with water vapour. In method A the vaporisation cylinders were kept in a water-bath at 120°F. and separated from the ports by a 6-ft. length of glass tubing (fig. 1A). In method B they were set immediately below the ports and kept at room temperature (fig. 1B). The vaporisation surface consisted of glass fragments in method A, and fluted filter paper in method B. The results in Table II show that the vapour from blood was more attractive than

TABLE II.

Attractiveness of the vapour from whole blood as compared with water vapour in the olfactometer.

Expt. No.	Number of mosquitos		Ratio	t
	Blood	Water		
Olfactometer Method A				
1	705	470	1.50	3.37
2	1164	747	1.56	5.05
3	859	826	1.04	0.11
4	1135	1093	1.04	0.24
Total	3863	3136	1.23	2.36
Olfactometer Method B				
1	177	34	5.20	2.87
2	387	50	7.74	5.74
3	53	37	1.44	0.85
4	30	27	1.11	0.46
5	513	558	0.92	0.24
Total	1160	706	1.64	2.06

water vapour. Although the difference proved to be statistically significant for each method, many of the individual experiments showed no significant difference. Additional comparisons were made by method A in which the vaporisation cylinders were held at 100°F. and 80°F.; in no case was a significant difference obtained.

The day-to-day variation in the response of mosquitos to blood exposed in petri dishes, in comparison with water, was therefore investigated. When an

uneven-aged population was tested in the large cage, attractiveness ratios of 1.44, 1.96 and 0.60 were obtained on the third, fourth and fifth days after the last blood-meal. An even-aged population of 150 females was then maintained on glucose in the small cage and tested daily by exposing whole blood in comparison with water, in petri dishes. The attractiveness ratios obtained (fig. 2) reached a

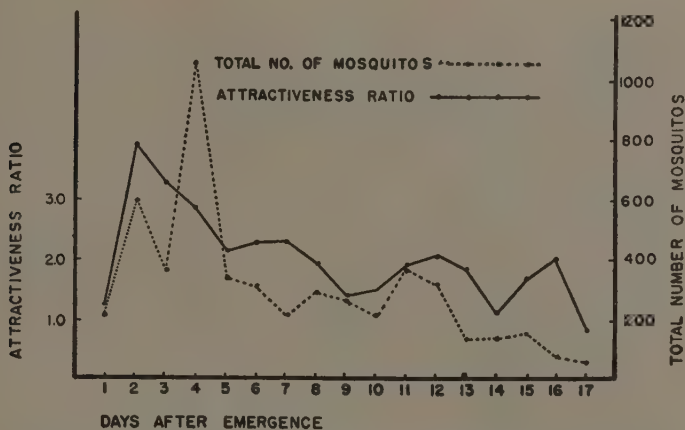


Fig. 2.—Attractiveness of whole blood to a population of mosquitos on consecutive days. Solid line, ratio between numbers at experimental port and at control port; dotted line, total number of mosquitos at both ports in a given time.

maximum of 3.84 on the second day after emergence, fell to a value of 2 by the eighth day, and thereafter remained between 2 and 1 until the 17th day, by which time the mosquitos showed little interest in biting a human hand or rabbit placed in the cage. The general reactivity of the mosquitos as measured by the total number of mosquitos coming to either dish (fig. 2, dotted line) showed a similar decline. Therefore the age of the mosquitos used in subsequent experiments was limited to the range from 3 to 14 days.

To elucidate the rôle in attractiveness played by the continual evolution of gaseous CO_2 from blood, the CO_2 -evolving factors were removed both singly and collectively. Bicarbonate was removed by adding 0.33 cc. of 33 per cent. BaCl_2 solution to each cc. of blood. Molecular CO_2 from other sources was removed by exhausting into a vacuum pump at 4 mm. Hg and 73°F . for 16 hours. Chemical tests made by passing CO_2 -free air over the treated blood and into saturated $\text{Ba}(\text{OH})_2$ solution showed that neither of the above treatments alone removed the ability of blood to evolve CO_2 , but that a combination of both treatments did so. It should be noted that the vacuum treatment could also remove volatile substances. Blood submitted to each of these treatments, and to a combination of both treatments, was then compared for attractiveness with washed corpuscles in petri dishes in the small cage. The results (Table III) show that neither BaCl_2 (which leaves free CO_2 in the blood) nor vacuum (which leaves the bicarbonate to evolve CO_2) remove the attractiveness of blood. In fact, the attractiveness ratios of 3.08 and 2.99, respectively, are even larger than the value of 2.34 obtained from whole blood in a similar comparison (see Table I). However, the use of BaCl_2 in conjunction with a vacuum reduces the attractiveness ratio to 0.96 as compared with washed corpuscles. It is therefore evident

that complete removal of CO_2 from all sources, as well as of other volatile factors, is accompanied by the elimination of the attractiveness of beef blood for these mosquitos.

Experiments were then conducted to ascertain whether the attractiveness removed from blood by the combination of BaCl_2 and vacuum could be restored

TABLE III.

Changes in the attractiveness of blood caused by treatments that remove different CO_2 -producing components.

Expt. No.	Number of mosquitos		Ratio	t
	Blood, treated BaCl_2	Corpuscles		
1	470	187	2.51	3.01
2	291	97	3.00	2.59
3	605	160	3.78	4.23
Total	1366	444	3.08	5.55
	Blood, vacuum-treated	Corpuscles		
4	865	195	4.44	2.63
5	1702	602	2.83	4.70
6	1206	464	2.60	2.78
Total	3773	1261	2.99	5.77
	Blood, BaCl_2 and vacuum-treated	Corpuscles		
7	217	345	0.63	2.41
8	343	285	1.20	1.44
9	173	137	1.26	0.69
Total	733	767	0.96	0.35

by the addition of gaseous CO_2 . Blood freed of CO_2 by these two processes was taken up in a glass syringe and 0.24 volumes per cent. of gaseous CO_2 was injected into it from a second syringe. Three hours later this sample of blood was compared for attractiveness with the original CO_2 -free blood, by exposure in petri dishes in the small cage. A total of three experiments (Table IV) showed that the reconstituted blood was 30 per cent. more attractive than the CO_2 -free blood and the difference was highly significant. In a second series of three experiments, the CO_2 -free blood had been treated with sufficient gaseous CO_2 to give it an emission rate of CO_2 approximately equal to that of whole blood, by exposing it to 16 volumes per cent. of CO_2 in a glass syringe for three hours. When compared with whole blood, this reconstituted blood was only 0.49 times as attractive as whole blood, the difference being highly significant (Table IV). Therefore the restoration of CO_2 only returns a fraction of the attractiveness of whole blood.

The possibility of increasing the attractiveness of whole blood by adding excess CO_2 was investigated by exposing it to a saturated atmosphere of CO_2 for three hours. When it was then compared with untreated whole blood in petri

dishes, the attractiveness ratio was 1.04, showing that the addition of excess CO_2 had not enhanced its attractiveness.

Two groups of experiments were undertaken with the modified olfactometer to determine the effect on attractiveness of passing the vapour from blood through a saturated solution of $\text{Ba}(\text{OH})_2$ by means of a fritted disc in a gas-washing

TABLE IV.

Changes in the attractiveness of CO_2 -free blood with the addition of gaseous CO_2 .

Expt. No.	Number of mosquitos		Ratio	t
	Blood, CO_2 -free; CO_2 added	Blood, CO_2 -free		
1	630	365	1.73	1.75
2	1057	847	1.25	1.94
3	1436	1184	1.21	1.48
Total	3123	2396	1.30	3.00
	Blood, CO_2 -free; CO_2 added	Whole blood		
4	189	605	0.31	2.81
5	290	665	0.44	1.65
6	399	510	0.78	0.40
Total	878	1780	0.49	2.38

bottle. This treatment removes all the CO_2 and may well remove other substances. The blood was exposed on a fluted filter paper in the vaporisation cylinder, which was separated from the port by the gas-washing bottle. This CO_2 -freed vapour was compared with normal blood vapour in one experiment and with water vapour in a second experiment (Table V). The air flow was regulated to 1 litre per minute at each port, which ensured the retention of all CO_2 by the barium hydroxide, while water permitted it to pass through; the air was removed after emission from the ports. The results showed that blood vapour from which the CO_2 had been removed was significantly less attractive than normal blood vapour, and not significantly more attractive than water vapour.

In an attempt at further characterising the attractive material in whole blood, it was submitted both to vacuum distillation and to desiccation. Blood was distilled at 98°F . and 4 mm. Hg into a receiver cooled by a mixture of acetone and dry ice; the open system led to a vacuum pump which operated continuously for 10 hours.

When the condensate was compared with distilled water on filter papers in petri dishes it proved to be significantly attractive, the full set of four experiments yielding an attractiveness ratio of 1.49 and a t_{31} of 2.31. This attractiveness was most marked upon initial exposure to the mosquitos, i.e., during the first four counts, made during the first eight minutes; by the time of the second four counts the attractiveness had disappeared. When the petri dishes were warmed to 88°F . or to 95°F . by being held on metal blocks warmed to those temperatures during the exposure period, the attractiveness was completely lost, even by the time the first counts were made, suggesting that the attractive principles were rapidly volatilised away at these temperatures.

The residue remaining from the vacuum distillation of blood was then compared with whole blood in petri dishes, and proved to be just as attractive as the whole blood (attractiveness ratio of 0.99 for a total of 3 experiments). When compared with washed corpuscles, in a total of three experiments, its attractiveness ratio was 2.40, and the attractiveness was highly significant ($t_{23} = 3.10$).

TABLE V.

The effect on the attractiveness of blood vapour of the removal of CO_2 by passage through $\text{Ba}(\text{OH})_2$ solution.

Expt. No.	Number of mosquitos		Ratio	t
	Blood vapour, CO_2 -free	Blood vapour		
1	842	1013	0.83	0.78
2	545	846	0.64	3.22
3	572	716	0.80	1.13
Total	1959	2575	0.76	2.35
	Blood vapour, CO_2 -free	Water vapour		
4	910	741	1.23	0.90
5	605	715	0.85	1.75
6	1222	988	1.24	0.90
Total	2737	2444	1.12	0.90

Therefore vacuum distillation must have removed a certain amount of a highly volatile component, but left behind in the blood the bulk of the attractive material.

Whole blood was then evaporated to dryness by exposing aliquots of 5 cc. on filter paper at 34°F . and 15 mm. Hg for 12 hours in a vacuum desiccator. When compared with washed corpuscles (similarly desiccated) in petri dishes, the attractiveness ratio for three experiments was 0.95. When the whole blood and the corpuscles were remoistened and compared in three experiments the attractiveness ratio for the former was 1.33, but the difference was not significant ($t_{23} = 1.45$). When the remoistened whole blood was compared with a suspension of washed corpuscles, it was no more attractive, the ratio for 3 experiments being 1.01. Therefore it is evident that desiccation removed the attractive material from whole blood, and remoistening did not return it.

Finally, attempts were made to discover whether an ether-soluble component of blood was attractive to adult *Aedes aegypti*. The plasma from 5 cc. of blood was shaken in a separatory funnel with three successive 5-cc. portions of ether at room temperature, which were evaporated to dryness on filter paper. This was then moistened with 5 cc. of water and compared with filter paper moistened with distilled water in petri dishes. For a total of three experiments, the attractiveness ratio was 0.85, showing that the moistened ether-soluble residue was not attractive to the mosquitos.

Discussion of Results.

The results of the experiments indicate that beef blood, prevented from clotting by heparin, is attractive to mosquitos. They stand in contrast to the findings of Howlett (1910) and Rudolfs (1922) that simple exposure of untreated

beef blood or human blood was unattractive to those species of the tribe Culicini tested by them. But they exactly parallel the finding of Reuter (1936) that defibrinated pig blood absorbed on filter paper is more attractive to *Anopheles m. atroparvus* than water on filter paper. In addition, the olfactometer experiments here reported with beef blood for *Aedes aegypti* parallel the results of Laarman (1955) obtained with rabbit blood for *Anopheles m. atroparvus*, a species to which beef blood is less attractive.

The experiments also provide definite indications that carbon dioxide is concerned in the attractiveness of beef blood to mosquitos. Removal of carbon dioxide from the vapour of normal blood reduced its attractiveness to that of water vapour. Blood treated so that it no longer emitted carbon dioxide attracted no more mosquitos than washed corpuscles (whereas whole blood attracted 2.3 times as many as washed corpuscles). During the treatment with vacuum other substances besides CO_2 could have been removed. Thus it was found that whereas readdition of CO_2 increased the attractiveness by 30 per cent., this is not nearly as much as the 130 per cent. increase of attractiveness shown by the whole blood over washed corpuscles. Indeed the blood reconstituted to emit CO_2 was only half as attractive as whole blood. This might well indicate the presence of another volatile attractive principle or principles that may be three times as potent biologically. When the attractiveness of blood was removed by treating it with BaCl_2 and a vacuum, probably other odorous substances were completely removed in addition to carbon dioxide.

Laarman (1955), whose olfactometer experiments on *Anopheles m. atroparvus* were as yet unpublished when the present investigation was in progress, employed dry substances to remove water vapour and CO_2 from the odour of a caged rabbit, namely silica-gel for water vapour and fine soda-lime for CO_2 . Thus he was able clearly to show an attractiveness for the rabbit odour, which he considers to derive from the blood by passage outwards through to the capillaries of the lung and skin, when the two factors of water vapour and CO_2 had been removed. The removal of CO_2 from moistened rabbit odour reduced the average attractiveness percentage from 92.4 to 87.6 (in the terms used in the present paper, from an attractiveness ratio of 12.2 to one of 7.1). In open-air experiments with the same species, van Thiel & Weurman (1947) found that, just as the addition of pig blood increased the attractiveness of a CO_2 -containing system by seven times, so the addition of CO_2 to a blood-containing system increased its attractiveness by seven times also. It would therefore appear that CO_2 is about as important as the other olfactory principles of blood for *Anopheles m. atroparvus*, whereas our experiments indicate it to be approximately one-third as important to *Aedes aegypti*.

Although there are strong indications that these other olfactory principles exist, they have yet to be characterised. In the present experiments they evidently were absorbed by passage through an aqueous solution of $\text{Ba}(\text{OH})_2$, and are removed by vacuum exhaustion for 18 hours. Searches for attractive compounds which occur in the blood, and may be expected to exude in sweat and insensible perspiration have hitherto proved negative or equivocal. Of a great number of materials tested against *Aedes sollicitans* and *A. cantator* by Rudolfs (1922), only a few, namely benzoic acid, dilute ammonia, phenylalanine, alanine, aspartic acid, cystine and haemoglobin proved attractive. However, Reuter (1936) found that the last six materials were unattractive to *Anopheles m. atroparvus*, and that formic, propionic, butyric, caproic and lactic acids were without attractiveness. DeLong & others (1949) found a slight attractiveness for lactic and propionic acids and a slight repellency for formic and acetic acids, to *Aedes aegypti*. In the tests made by Brown, Sarkaria & Thompson (1951), acetic and lactic acids, ammonia and trimethylamine alike proved unattractive to this mosquito, as also did such alcohols, aldehydes and esters as are known to be

attractants for the house-fly. Reuter (1936) had found indole and skatole to be as unattractive as ammonia to *Anopheles m. atroparvus*. Rudolfs (1922) also found that peptone was attractive; it not only exerted a high degree of attraction but also induced feeding reactions. The report of Schaerffenberg and Kupka (1951) is the most promising evidence that olfactory principles do exist in the blood, which are active in such high dilutions that CO_2 could not be involved as a factor.

The experiments here reported may be taken as evidence that the CO_2 in beef blood is a definite element in its attractiveness, though probably not the most important one; and that the attractiveness of a warm-blooded animal to *Aedes aegypti* may be partially attributed to the CO_2 transpired through the skin from the cutaneous capillaries. This is substantially the view advanced by van Thiel (1937) for *Anopheles m. atroparvus*, who considered that it may be reinforced by a specific odorous substance in the blood. This substance or complex still awaits characterisation.

Summary.

The responses of females of *Aedes aegypti* (L.) to beef blood have been investigated. Paired comparisons were made of the numbers of mosquitos attracted to test and control materials.

Vapours emanating from the heparinised blood were found to be significantly more attractive than water vapour in an olfactometer. When the blood vapour was passed through an aqueous solution of $\text{Ba}(\text{OH})_2$ this attractiveness disappeared.

Exposure of heparinised blood on filter paper proved it to be two to four times as attractive as water or washed corpuscles suspended in saline. Plasma proved to be 4.7 times as attractive as water, indicating that the attraction of blood lies entirely in the plasma.

Treatment of blood with BaCl_2 and a vacuum, thus rendering it incapable of evolving CO_2 , removed its attractiveness to mosquitos. Treatment with vacuum alone, which may be expected to remove the volatile substances, did not completely remove the attractiveness; nor did the treatment with BaCl_2 alone, which removes the bicarbonates in the blood.

The attractiveness of blood was completely removed by desiccation and was not returned by remoistening. No attractive substance was isolated in an ether extract of blood.

When CO_2 -freed blood was treated with gaseous CO_2 its attractiveness was significantly increased. When the treatment was such as to restore the normal CO_2 -producing capacity, the attractiveness was one-half that of whole blood. The attractiveness of whole blood was not increased by adding excess CO_2 .

It is concluded that the CO_2 in beef blood is a definite element in its attractiveness, though probably not the most important one.

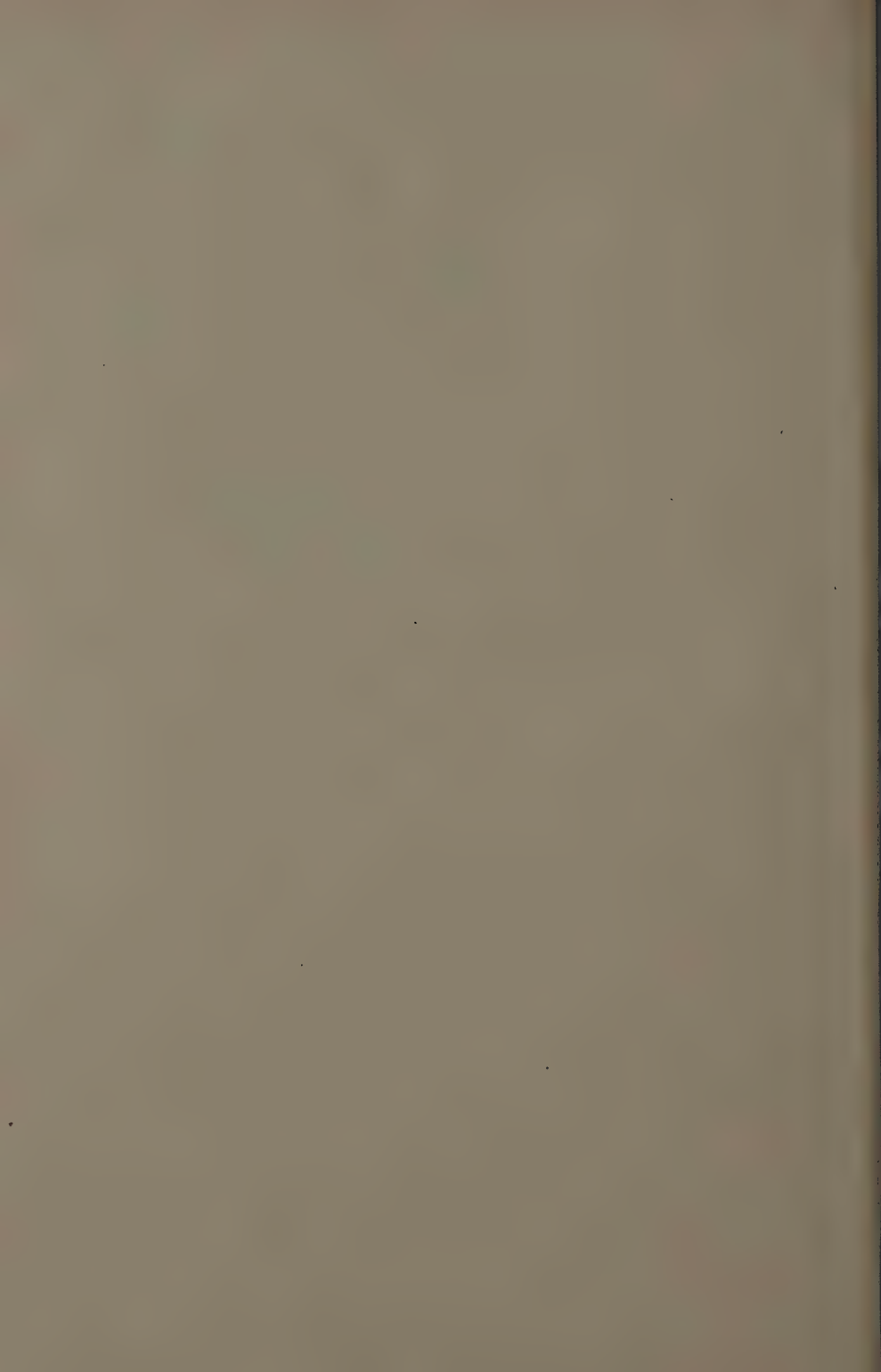
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THE DISTRIBUTION OF THE STORAGE SPECIES OF *CRYPTOLESTES* * (COL., CUCUJIDAE).

By R. W. HOWE and L. P. LEFKOVITCH.

E.M.

The study of the distribution of insects infesting stored produce has limited significance since world trade assists them to become cosmopolitan. Occasionally, however, a species is able to change its distribution or local economic importance as a result of some environmental change. Thus *Ephestia kuehniella* Zell. became a serious pest in flour mills throughout the temperate regions of the world about 1880 following the introduction of artificial power into mills; *Ptinus tectus* Boield., a species restricted to humid temperate areas in the southern hemisphere was killed by heat during the journeys across the tropics until the introduction of fast ships during the present century enabled it to become abundant in both hemispheres; and very recently *Trogoderma granarium* Everts has spread its range to many of the warm dry areas of the world, probably as a result of changes in trade routes during the last 15 years.

A study of the distribution and biological characteristics of an economic species may therefore give warning, as was given for *P. tectus* (Howe & Burges, 1953), of the possibility of its spreading or increasing its importance.

The distribution of the various species of flat grain beetles is very imperfectly known because they are difficult to identify with confidence. *Laemophloeus* is a heterogeneous group comprising over three hundred species, but those commonly found in stored produce fall into a uniform group, *Cryptolestes*, which merits generic status (Steel & Howe, 1955).

Existing keys to these species are unreliable and it is very likely that many of the published records are based on misidentified specimens. Frequently also, identification is not carried to a specific level. The present paper is based upon the identification by the authors of some 3,500 samples of *Cryptolestes*, mostly collected by inspectors of the Infestation Control Divisions of the Department of Agriculture for Scotland, and the Ministry of Agriculture, Fisheries and Food. The essential characters used in identification were the genitalia of either sex, although the species can be recognised by combinations of external characters. The genital characteristics are figured by Reid (1942) for *C. ferrugineus* (Steph.), *C. minutus* (Ol.) and *C. turcicus* (Grouv.), by Steel & Howe (1952) for *C. pusilloides* (Steel & Howe) and by Steel & Howe (1955) for *C. ugandae* Steel & Howe. Similar diagrams are given here (figs. 1 & 2) to establish the identity of the species which we call *C. spartii* (Curt.).

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The records considered fall into three categories. Firstly there are those of insects collected in various countries which can normally be accepted as genuine evidence that the insects are now established in these countries. Secondly there are records of insects collected aboard ships on produce from various countries. These may include species acquired on the ship from residual populations and from other cargoes, but the records as a whole may be used to show the world distribution of species, since regular cross-infestation of a particular product from a particular place is unlikely. Finally there are the data from samples collected in Britain from warehouses and mills. These show to some extent how imported species are spread within importing countries and also the association between species and various types of produce and premises.

* Often called *Laemophloeus*.

Species collected in Countries of Origin.

The specimens considered in this section were collected mainly by Dr. J. A. Freeman of the Infestation Control Division when travelling on behalf of the Organisation for European Economic Co-operation, or by Messrs. D. W. Hall and G. A. Haswell of the Pest Infestation Laboratory.

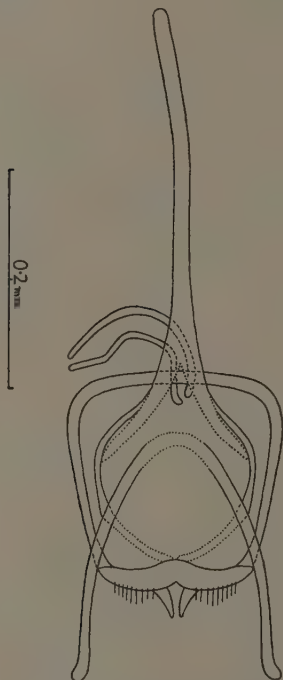


Fig. 1.—Sclerotisations associated with the male genitalia of *Cryptolestes spartii*.

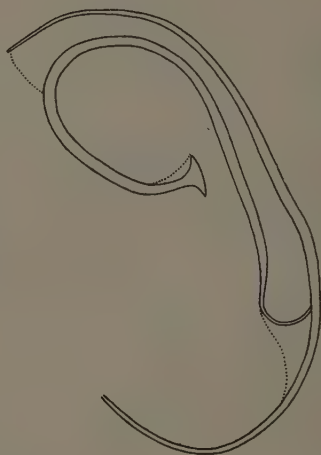


Fig. 2.—Spermatheca of female of *C. spartii*.

Australia.

Several samples of insects collected on sorghum in northern Queensland, and on wheat in New South Wales and Victoria have been sent to us for identification by Mr. S. W. Bailey of the Commonwealth Scientific and Industrial Research Organisation. From these it is clear that *C. ferrugineus*, *C. minutus* and *C. pusilloides* are all common in these States. Mr. Bailey failed to find *C. turcicus* in spite of making a special examination of 7 flour mills. Davidson (1940) stated that he had never seen *C. turcicus* in South Australia. *C. pusilloides* was found in wheat porridge and in dried mushrooms bought from shops in Sydney, N.S.W.

South America.

All the specimens identified from South America except British Guiana (see under Caribbean), were collected by Dr. Freeman on a visit to Brazil, Uruguay and Argentina in October and November 1954.

Both *C. ferrugineus* and *C. minutus* were collected in Uruguay and Argentina on wheat and sunflower seed. *C. minutus* was also taken in a flour mill in Rio de Janeiro, Brazil. *C. turcicus* was found in flour mills in Montevideo, Uruguay and Buenos Aires, Argentina. No specimens of *C. pusilloides* were collected.

Caribbean.

Mr. Hall visited the Commonwealth Caribbean territories between April and June 1955. *C. minutus* was the most abundant and widespread species in this area, being taken 13 times in British Honduras, six times in British Guiana, eight times in Trinidad, four times in St. Lucia, twice in Jamaica and once in Barbados. Twenty of these occurrences were on rice or paddy and five on maize, the other records being on imported alfalfa meal, copra, flour, maize meal, oats, sorghum and wheat. *C. ferrugineus* was taken three times in British Honduras, twice in Jamaica and once in British Guiana; three of these records were on rice, two on maize and one on cocoa. *C. pusilloides* was found once, in Trinidad, on rice imported from British Guiana.

North America.

Both *C. ferrugineus* and *C. minutus* are widespread and abundant in North America. *C. ferrugineus* appears to be the commoner species in Canada (MacNay, 1955). During August and September 1954, Dr. Freeman collected *C. ferrugineus* six times on wheat, maize and copra but took *C. minutus* only once, at a grain elevator at Houston, Texas. He also collected *C. turcicus* twice on maize at Ames, Iowa. This latter species has been taken from the milling stream of flour mills in Buffalo, N.Y., by Dr. R. O. Rilett (see Rilett & Weigel, 1956) who kindly sent us specimens to examine. It is also present in the collection of the University of Minnesota, the specimens having been taken in a Minneapolis flour mill in 1930. This collection also includes a single specimen of *C. pusilloides* taken in Minneapolis in 1941. Some specimens of *C. minutus* in the British Museum (Natural History) were collected in Mexico by Sharp (1899) and were described by him as a new species, *Laemophloeus pauper*, which has since been shown (Steel & Howe, 1952, p. 88) to be a synonym of *C. minutus*.

The Mediterranean area.

Dr. Freeman visited Morocco, Algeria, Tunisia, Greece and Turkey between February and April 1955. He collected *C. ferrugineus* once from a silo in Morocco, once each from wheat and barley in Algeria, four times from wheat in Tunisia and six times from wheat and once from rye in Turkey. *C. minutus* was found once on wheat, and once each in a silo, a warehouse and a flour mill in Morocco, twice in wheat and once in barley in Tunisia, in a flour mill in Greece and three times on wheat and once on rye in Turkey. *C. spartii* and *C. turcicus* were found in 16 flour mills. Zacher (1940) has recorded *C. spartii* from Egypt. Dr. Freeman found it in three mills in Morocco, one in Tunisia, and two each in Greece and Turkey. He collected *C. turcicus* from two mills in Morocco, Algeria and Greece and from three in Tunisia and Turkey.

Africa south of the Sahara.

All the Commonwealth territories in this region have been visited by Mr. Hall, the eastern countries have been revisited by Mr. Haswell, the Gambia by Mr. A. A. Green and Ghana by Miss P. M. Davey, all of the Pest Infestation Laboratory. *C. minutus* is the most widespread species in tropical Africa. It is not, however, found in the dry areas of northern Nigeria (Howe, 1952), although it is found in the more humid south (Cotterell, 1952). The products from which *C. minutus* and *C. ferrugineus* were collected in Africa are listed in Table I. In December 1955, Mr. Haswell found these two species in only three territories,

Tanganyika, Kenya and Southern Rhodesia. The latter two were the only territories in which Mr. Hall did not collect them during his tour from January to March 1952. All the produce found infested in Zanzibar was imported either from Tanganyika or south-east Asia.

In February 1952, Mr. Hall collected *C. ugandae* on cassava, groundnuts, maize and sorghum at Kisoko, Soroti and Mbale in Uganda, where it appeared

TABLE I.

Products from which *Cryptolestes minutus* and *C. ferrugineus* have been identified from African countries.

Country	Species	
	<i>C. minutus</i>	<i>C. ferrugineus</i>
Gambia	—	groundnuts
Ghana	beans, cassava, cocoa, cowpeas, groundnuts, maize, Nere seed, rice	—
Nigeria	beans, cocoa	beans, groundnuts, sorghum
Kenya	beans, maize, sorghum, wheat	maize
Uganda	cassava, groundnuts, maize	—
Zanzibar	rice, sorghum	cassava, flour, rice, sorghum
Tanganyika	maize, rice, sorghum	maize, rice, sunflower seed
Nyasaland	beans, maize, wheat	maize, wheat
N. Rhodesia	maize, wheat	wheat
S. Rhodesia	maize	maize
Portuguese E. Africa	groundnuts	groundnuts
Union of S. Africa ..	maize, wheat	wheat

to be relatively common. In December 1952, he collected a few specimens on cowpeas at Ibadan, Nigeria, and on sorghum at Gonja, Ghana. In April 1955, Miss Davey found this species on sorghum in Ghana at Bolgatanga and Bawan. This species is obviously of limited distribution and laboratory experiments show that it needs a high humidity if it is to multiply (Lefkovitch, 1957).

C. pusilloides was found by Mr. Hall in 1952 on buckwheat, maize and wheat in South Africa and on wheat in Northern Rhodesia. Mr. D. J. W. Rose, of the Department of Research and Specialist Services, S. Rhodesia, sent specimens from maize from Southern Rhodesia late in 1953 and Mr. Haswell collected this species on groundnuts in Swaziland in 1955. He also found *C. pusilloides* in Tanganyika on rice and wheat and on the walls of a warehouse. Probably this species has spread from the south, possibly by means of the ships from South African ports which call also at East African ports. Such a conclusion must, however, be very tentative, because the records of the two common species of *Cryptolestes* were so markedly different in the collections made by Messrs. Hall and Haswell. *C. pusilloides* was absent from a sample from maize collected in Nyasaland by Dr. K. F. Salmond, of the Department of Agriculture, Nyasaland, and sent for identification, but was found in Uganda in December 1956 by Mr. J. C. Davies, of the Department of Agriculture, Uganda. *C. minutus* was extremely abundant in both samples with a few *C. ferrugineus*.

C. turcicus, according to specimens in the British Museum, has been found on flour in the Belgian Congo and has recently been sent to us from Uganda by Mr. Davies.

Conclusions.

The above records include no specimens collected in Asia and they are sparse for most other areas. Most of Europe is excluded because it is an importing

area. It is fairly clear that the commonest and most widespread species were *C. minutus* and *C. ferrugineus*, which are often termed cosmopolitan. *C. minutus* was most abundant in the more humid tropical areas whilst *C. ferrugineus* extended into drier and cooler areas. *C. pusilloides* is known only in the three southern continents but may be spreading. *C. ugandae* is very restricted in distribution. *C. spartii* was found only in the flour mills of countries bordering the Mediterranean. *C. turcicus* was found in both North and South America in addition to the Mediterranean area. With the exception of two records on stored maize in the U.S.A., it occurred only in the flour mills. The specimens in the British Museum from the Belgian Congo and those collected in Uganda are not sufficient evidence to conclude that *C. turcicus* is an established breeding species in that part of Africa, since they may have been imported with foodstuffs from Europe. This species has, however, been recorded in Belgium as having occurred on maize imported from the Congo.

Species collected from Ships at British Ports.

Since November 1950, the inspectors of the Infestation Control Divisions have collected specimens of *Cryptolestes* seen in ships whenever this has been possible. From over 1,600 identifications of collections of specimens from ships it is possible to draw some fairly reliable conclusions on the world distribution of some of the species.

These conclusions are limited by the origins of the products imported and inspected and by the nature of the produce. It is not possible, for instance, to deduce how any species varies in abundance from one area to another because

TABLE II.

Countries of origin of *Cryptolestes* spp. collected on ships at British ports, with numbers of records for each species.

Country	Species				
	<i>C. ferrugineus</i>	<i>C. minutus</i>	<i>C. pusilloides</i>	<i>C. turcicus</i>	Others
Australia	134	56	111	3	—
Argentina and Uruguay (=Plate)	171	108	81	2	—
Brazil	10	19	10	—	—
Rest of S. America	5	2	—	—	—
West Indies	—	4	—	—	—
U.S.A.	47	17	—	23	—
Canada	69	5	2	—	—
Mediterranean Europe	12	7	5	5	2
French N. Africa	15	1	—	—	—
Middle East	61	9	1	1	—
Sudan	5	—	—	—	—
West Africa	124	63	1	2	5
East Africa	54	68	11	1	2
Union of S. Africa	33	15	60	—	—
India and Ceylon	25	7	1	—	1
Burma and Siam	78	23	2	—	1
Malaya and Singapore	15	18	1	1	3
China	7	2	—	—	—
U.S.S.R.	12	2	1	—	—
Northern Europe	12	6	—	1	—
Others	3	2	—	2	—
Total	892	434	287	41	14

the data available are based only upon the specimens collected and identified and include no information as to how often *Cryptolestes* spp. were absent, or if present, were not collected. Over the period 1944-46 one-quarter of cereal cargoes was infested by *Cryptolestes* (Freeman, 1948). For the year 1953 the Infestation Control Division has prepared summaries of the reports for all the cargoes inspected. These summaries show that *Cryptolestes* spp. were found on about 9 per cent. of cargoes and that they were identified to species by us for about two-thirds of these records. The great majority of the records of *Cryptolestes* in 1953 were for whole cereals and cereal products, about 13 per cent. of such cargoes being infested. About 8 per cent. of cargoes from West Africa, mainly oilseeds, were infested by *Cryptolestes* spp. Less than 3 per cent. of cargoes of oilseed produce from other areas, and only about 1 per cent. of cargoes such as legumes and dried fruits were infested. These beetles were not found on cargoes of animal origin such as meatmeal. During 1953, the cargoes imported from Argentina, Australia (New South Wales, Queensland and Western Australia), Burma, the west-coast ports of Canada, the Middle East (Iraq, Lebanon and Syria), Nigeria and East Africa were frequently infested by *Cryptolestes* spp. Only one cargo of the many inspected from the east-coast ports of Canada carried these beetles.

In Table II, the species identified from ships are listed according to the origin of the cargo on which they were found. Just over half were *C. ferrugineus*, over one-quarter *C. minutus*, and nearly one-fifth *C. pusilloides*. *C. turcicus* was found on only 2 per cent. of cargoes. The column headed "others" includes four species not yet identifiable as described species, one of which has been found on five occasions, and also two records of *C. spartii* and three of *C. ugandae*.

From Table II it is reasonable to conclude that *C. turcicus* occurs in the U.S.A. and that *C. pusilloides* occurs in Australia, South America and South Africa. The records of the latter species for East Africa may be due to cross-infestation in ships which carry produce from both South and East Africa. It may be concluded also that *C. minutus* is probably the commonest species in East Africa and perhaps Malaya, the West Indies and Brazil and that it is common in Argentina, Australia and West Africa, although not so common as *C. ferrugineus*.

Some interesting points arise from a closer consideration of the data from each country. Thus *C. pusilloides* (Table III) is associated with sorghum both from Australia, no matter from which State it was exported, and from South Africa. *C. pusilloides* is also associated with maize from South Africa but is seldom found on Brazilian maize, although it was found frequently on rice from Brazil. Wheat is infested by all three species in the southern hemisphere with *C. ferrugineus* usually being found twice as often as *C. minutus* and *C. pusilloides*. This is true also for milling products from South America, but *C. pusilloides* was the species most often found on Australian milling products.

United States produce infested by *Cryptolestes* was almost entirely wheat and maize. For each, *C. ferrugineus* formed about half the records and *C. minutus* and *C. turcicus* about one-quarter each. From Canada, only wheat was infested, and almost all the cargoes found infested by *Cryptolestes* were exported through the west-coast ports. Not only does the west coast of Canada have a milder winter than the east coast, allowing more insects to survive, but also the trade route to Europe is longer and passes through a sub-tropical region favourable to the multiplication of these insects.

Little of interest can be deduced from the data for the part of Europe close to the Mediterranean (Iberia, Italy, the islands, Balkans and Rumania) because the produce infested was varied and the total records small. In addition, this area imports infestible goods and probably has by now acquired alien species such as *C. pusilloides*. It is interesting, however, to note that *C. turcicus* and

C. spartii were found thrice and twice, respectively, on Italian wheat bran. *C. ferrugineus* forms almost the entire record for French North Africa and the Middle East (Libya to Turkey including Iraq and Iran) which is surprising in view of Dr. Freeman's captures of *C. minutus* in North Africa. Of the ten records of *C. minutus* on ships from these areas, three were on sorghum and seven on bran. Of the 76 records of *C. ferrugineus*, 30 were on bran and 31 on barley.

TABLE III.

The infestation by *Cryptolestes* spp. of produce from the southern hemisphere.

Produce		Species		
		<i>C. ferrugineus</i>	<i>C. minutus</i>	<i>C. pusilloides</i>
Australia:	Wheat	102	36	37
	Wheat products	3	5	13
	Sorghum	8	12	50
	Other cereal seeds	14	3	9
S. Africa:	Maize	13	11	35
	Sorghum	4	1	16
	Bran and pollards	15	3	5
Brazil:	Rice	—	2	8
	Maize	9	13	1
Argentina and Uruguay (=Plate)	Maize	26	25	3
	Wheat	58	39	41
	Barley	8	6	5
	Wheat products	69	35	29
S. American oil seed products		7	3	3

The predominance of *C. ferrugineus* in these areas and in the Sudan may indicate that this species is the one that can best stand dry conditions. This is supported by the data for West Africa, because groundnuts, the main produce of the dry north of this area, are little infested by *C. minutus* (Table IV), although all the ports of this area are in humid regions. Palm kernels from this area are also infested mainly by *C. ferrugineus*, but *C. minutus* is the species normally found on cocoa beans. *C. minutus* was slightly more usual than *C. ferrugineus* on most East African produce. *C. pusilloides* was found eight times on maize from East Africa.

TABLE IV.

The infestation by *Cryptolestes* spp. of produce from West and East Africa.

Produce		Species		
		<i>C. ferrugineus</i>	<i>C. minutus</i>	<i>C. pusilloides</i>
West Africa:	Groundnuts	62	3	—
	Palm kernels	23	1	—
	Cocoa beans	15	41	—
East Africa:	Cottonseed products	18	22	—
	Maize	19	17	8
	Sorghum	6	7	1

An unidentified species was taken five times on cargoes from southern Asia, three times on Malayan illipe nuts, once on desiccated coconut from Ceylon and once on Burmese rice. *C. ferrugineus* was predominant on all produce from Burma and Siam. On rice, for instance, *C. minutus* was taken only 14 times as against 42 for *C. ferrugineus*. The total number of records for Malaya is small. On illipe nuts there were five records of *C. ferrugineus* and six of *C. minutus* and on copra there were seven of *C. ferrugineus* and three of *C. minutus*, but the latter species was the commoner on rice and sago.

Summing up the records of the species found in ships, the conclusions are, once more, that *C. pusilloides* is a southern-hemisphere species which may be spreading, and that *C. ferrugineus* is the species usually found in the more temperate or drier areas, while *C. minutus* is the commoner species of the hotter humid areas. Associations with a product are most likely to be linked to the area of origin of the product and the climate there. There are possible exceptions, such as the association of *C. ferrugineus* with palm kernels, which, for any given relative humidity, have a lower moisture content than many other products. The restricted occurrence of *C. turcicus* corresponds extremely well with Dr. Freeman's collecting, being found frequently only on United States produce. *C. spartii* is also very rare in imports.

Species collected in Warehouses and Mills in Great Britain.

Most of the infestations of *Cryptolestes* found in storage premises in Britain originate from individuals imported with produce, but two species, *C. ferrugineus* and *C. spartii*, are known to occur in the open in Britain (Allen, 1950; Massee, 1952). The former was the only species of *Cryptolestes* commonly identified by us from farm stores (Table V). It was often collected on home-grown barley,

TABLE V.

The numbers of records of *Cryptolestes* spp. collected in mills and storage premises in Great Britain.

Type of premises	Species				
	<i>C. ferrugineus</i>	<i>C. minutus</i>	<i>C. pusilloides</i>	<i>C. spartii</i>	<i>C. turcicus</i>
Farms	91	1	1	—	—
Buffer depots ..	87	26	6	—	4
Warehouses	349	138	58	4	19
Flour mills	68	17	3	38	544
Other mills . . .	64	11	3	27	25
Maltings	15	1	1	—	1

oats or wheat in store, but probably reached the farms on imported produce. On the whole it is more likely to spread from farm granaries to outdoor habitats than in the reverse direction. In a survey of farms made in 1938-40 (Howe, 1951), *C. ferrugineus* was found only twice. *C. ferrugineus* was almost the only species found in maltings and even this species was not common. Its presence is probably due to the high proportion of home-grown barley used in malting. Even in ordinary storage premises, *C. ferrugineus* was by far the commonest species, accounting for two-thirds of the records, with *C. minutus* forming another

quarter. In mills, however, *C. turcicus* was the common species, while *C. spartii* was found rather more often than *C. minutus*. Nearly all the specimens of *C. turcicus* and *C. spartii* were collected from the milling machinery (Table VI)

TABLE VI.

Site of collection of *Cryptolestes* spp. in mills of all kinds in Great Britain.

Site	Species				
	<i>C. ferrugineus</i>	<i>C. minutus</i>	<i>C. pusilloides</i>	<i>C. spartii</i>	<i>C. turcicus</i>
Machinery	8	5	—	45	515
Fabric of mill	22	3	—	6	17
Fabric of silo	19	5	2	1	6
Milling products	9	—	—	2	9
Spillage, sweepings, sacks, (grain and flour) ..	17	2	—	6	14
Wheat	23	5	3	2	5
Other cereal seeds	23	5	1	—	3
Oil seeds & beans	11	3	—	—	—

with some from spillage under the machines or from the fabric of the mill itself. *C. turcicus* was also collected occasionally on flour, empty sacks and wheat stored in flour mills. *C. ferrugineus* and *C. minutus* were mainly collected on wheat and on other raw materials in the storage sections.

The general pattern of infestation in storage premises, including the storage sections of mills, is consistent with that of the imported goods inspected in them. Thus *C. pusilloides* was found 49 times on produce known to have been imported from Brazil, Argentina, South Africa or Australia and only eight times on produce from elsewhere, with 12 records from the fabric of buildings or from goods of unknown origin. This shows that cross infestation by this species does occur in Britain but is not extensive. *C. minutus* also shows the import pattern of dominance over *C. ferrugineus* on cocoa from West Africa. It was also the commonest species on East African produce and was often found, though less commonly than *C. ferrugineus*, on Australian and South American produce. A surprisingly large number of records of both species, 61 of *C. ferrugineus* and 21 of *C. minutus*, were made for Canadian produce. This may be due to the tendency to hold Canadian wheat in store for long periods, so that it acquires insects by cross infestation, and also has the chance to heat and build up large populations. The five records of *C. pusilloides* on Canadian wheat are evidence of cross infestation. British conditions appear to suit *C. ferrugineus* better than the other species, for there is an increase in the proportion of this species in records from storage premises to 66 per cent. as compared with 53 per cent. on ships. *C. minutus* is often associated with heating grain. The infestation of mill machinery is not related to current importations.

Discussion.

The conclusions which may be drawn from the three kinds of samples discussed above are consistent. *C. ferrugineus* is quite clearly established in

Australia, South and North America, Africa, Asia Minor and Europe. It was also found in Central America and the West Indies, but its presence there may be due, in part at least, to imported goods. Import records suggest that it must be abundant in both the temperate and tropical parts of Asia, although there appear to be no clear records of this species having been collected in Asia except on Army stores (Davis, 1947) which were probably of Australian, American or European origin. There are records of *C. ferrugineus* for New Zealand (Belton, 1951), and Peru (Wille, 1934), but none for the islands in the Pacific.

Solomon & Adamson (1955), show that this species is more cold-hardy than *C. minutus*, *C. turcicus* or *C. pusilloides*, so its more extensive distribution in cooler areas is to be expected. No direct comparison of the biology of the species has yet been made, but the work of Rilett (1949) on *C. ferrugineus* and Davies (1949) on *C. minutus* does support the impression that low humidity, by increasing both mortality and the developmental period, affects *C. minutus* more severely than *C. ferrugineus*. There is also in these papers some indication that at 90 per cent. relative humidity and tropical temperatures, *C. minutus* increases the more rapidly. This corresponds with the observation that *C. minutus* is the commoner species in the West Indies, Brazil and the wetter parts of tropical Africa and probably the monsoon areas of Asia. It is also found, though less frequently than *C. ferrugineus*, in the cooler or drier parts of South and North America, Europe, Africa, Asia and Australia. It is the species usually recorded for India (Trehan & Pingle, 1948), China (Hsiu, 1936), Hong Kong (Herford, 1939), Fiji (Lever, 1943) and Bermuda (Ogilvie, 1928). Solomon & Adamson (1955) show that it is very susceptible to cold and so is unlikely to survive the winter in much of the temperate zone unless protected by heated produce or premises. It is, however, frequently caught in the open in summer in the U.S.A. (Schwitzgebel & Walkden, 1944). In North America this species has been found in the bran stream of flour mills (Watters, 1956). There are a few records in the present paper of the discovery of this species in mill machinery in Britain, Brazil, Morocco and Greece. This species and *C. ferrugineus*, which was also found in mill machinery on a few occasions, both enter flour and provender mills on cereals and have ample opportunity to invade the flour stream, so it is interesting to note this comparative scarcity in the habitat.

C. turcicus, on the other hand, seldom enters a flour mill on grain but is almost always present in the mill machinery in Britain (see also Williams, 1950). It also occupies this habitat in North Africa, Greece, Turkey, northern Europe, Argentina and Uruguay and in U.S.A. It is probably a native of Turkey (Grouvelle, 1877) and may have entered flour mills in the Mediterranean area by flight. Since it is relatively cold-hardy (Solomon & Adamson, 1955) even an occasional cargo of milling produce imported from the Mediterranean into northern Europe, the U.S.A. or any other temperate country could easily result in the establishment of the species in these countries. Presumably the food and environment in the machines of flour mills are such as to give this species some advantage over other species of *Cryptolestes*. There is no simple way at present to account for this species occurring in cereals only from the U.S.A. among the countries exporting to Britain. Unfortunately also, there is no way of discovering how long this species has been present in any part of its distribution, since it is so easily confused with the more widespread *C. minutus*. It was first recorded in England in 1925 by Joy, who found four adults on the window-sill of a flour mill. It may well have reached the U.S.A. at about the same time.

Another interesting feature is the persistence of *C. turcicus* in flour mills in spite of the regular use of fumigation and other control methods. This may suggest that the fumigations do not achieve complete control of the insects, but Freeman (1953) attributes reinfestation principally to empty flour sacks returned from bakeries and partly to imported flour bought for blending. It is possible

that *C. turcicus* also enters mills on wheat from the U.S.A., indeed this may have been the original source rather than imported Mediterranean flour. The greater part of the grain seen during a survey made in 1938-40 (Munro, 1940) was from the U.S.A. or the Plate, and most of the *Cryptolestes* not identified to species were found on these products. Again in 1944-46, the main sources of *Cryptolestes* were U.S.A., Brazil and the Plate (Freeman, 1948). Since the relative volume of imports from the U.S.A. is now less, and if the presence of *C. turcicus* on wheat from U.S.A. is of long standing, the proportion of *C. turcicus* introduced into mill silos may have been much larger in the past than at present. *Cryptolestes* spp. were common in British flour mills and provender mills in 1938-40, but it was not until 1950 that Williams stated that the species found in the flour stream of British mills was *C. turcicus*.

C. spartii, apart from two records from Italian bran in ships and one from imported nuts in a warehouse, was found only in mills. In flour mills it was considerably less common than *C. turcicus* and from casual observation it seems to be found usually in the coarser breaks of the mill or in parts of the mill where physical cleaning is very infrequent. In mills using other raw materials, such as maize, rice or soya beans, *C. spartii* was commoner than *C. turcicus*. The only areas in which this species is certainly established are northern Europe and the Mediterranean area. Judging from Dr. Freeman's records in flour mills in the Mediterranean area, 8 of *C. spartii* and 12 of *C. turcicus*, *C. spartii* is more abundant in that area than in Britain. Thus *C. spartii*, though rare in imports, may have been introduced into British mills on some mill product from that area. The records we have for Britain are equally divided between port and inland areas, and the latter include large town and small village mills. The outdoor form (see p. 802) of this species is much darker than the mill form, but it is not known whether the colour difference is due to food and environmental conditions. Neither is it known whether either form can survive in its less usual habitat, nor if the forms can interbreed, but it seems possible that the outdoor form could invade mills in the comparatively favourable climate of the Mediterranean.

The present known distribution of *C. pusilloides* (see p. 799) is clear-cut but difficult to explain. It was not recognised as a distinct species until 1944-45, when it was twice received at the Pest Infestation Laboratory in three months. Little more than a year later it was again found, this time by Mr. W. O. Steel, in grain purchased by the Hawthorndale Laboratories, Bracknell (Steel & Howe, 1952). Otherwise, so far as is known, it has been collected only in Minnesota, U.S.A., in 1941, by Dr. F. Zacher in Germany in 1951, and as mentioned in this paper. Since the species has been readily recognised as distinct by three workers independently, it seems unlikely that it entered stored products handled by the shipping trade much before 1944. If so, its origin and entry into this trade is interesting. The two earliest records in Britain were on wheat which had acquired the species by cross infestation, pointing to its origin in produce imported prior to 1946 and probably stored for some time. At this time it was known that the species was found in South Africa. By 1951, when material of the genus *Cryptolestes* had been identified regularly by us for a few months, it was evident that the species was then also present in Australia and in Brazil, especially in the rice-growing area. It was not until late 1953 that Argentinian produce was found to be considerably infested by this species—this coincided with a resumption of wheat imports from this area. Kessel (1926), in an extensive paper and key on the genus *Laemophloeus*, *sens. lat.*, published in Brazil, does not mention a species which can be interpreted as *C. pusilloides*, so it is quite likely that *C. pusilloides* has only recently invaded storage premises in Brazil. South Africa has imported wheat from Australia, and, in fact, Mr. Hall found this species on Australian wheat in South Africa. It is possible that this species is Australian and was able to multiply in stored grain during the

long-storage period of 1939-44, and was spread to other parts of the southern hemisphere by the irregular trade routes of this period. Alternatively, it could have been present originally in all three continents and have multiplied in all with the wartime storage of grain. In any event, it is not likely to have reached Britain directly from Australia in 1944-46, for few if any grain cargoes were imported into Britain from Australia at that time (Freeman, 1948).

C. pusilloides is not cold-hardy (Solomon & Adamson, 1955) and shows little sign of becoming established in Britain, although freely imported. It does, however, seem to be spreading into East Africa and must have the opportunity of invading south-east Asia and the West Indies. It may also spread, by trade from Mozambique, to Portugal, where it might be able to survive.

The final species, *C. ugandae*, is of very limited distribution in Africa and has scarcely entered into trade. A laboratory study of its life-cycle (Lefkovitch, 1957) shows that it needs an extremely high humidity. This is sufficient to account for its limited distribution. It is hoped that laboratory work with the other five species will help to explain some of the differences in distribution noted in this paper.

The small number of unidentifiable species seen are probably strays acquired from timber forming part of the cargo of ships. The species found on illipe nuts may, however, be truly associated with them, feeding either directly on the ripe nut on the tree or after it has fallen, or preying on other insects eating the nut.

Several other species have been recorded in the literature as found on stored produce. *Laemophloeus emgei* Rtt. is frequently mentioned and is described as being similar to *C. ferrugineus*, although Kessel (1926) uses a very clear distinguishing character in his key. All the specimens alleged to be *L. emgei* from stored products that we have been able to examine have been *C. ferrugineus*.

The other species of the genus *Laemophloeus*, *sens. lat.*, recorded in the literature on stored products are either strays or predators in a habitat not very different from that under bark. Thus Downes (1950) records *L. punctatus* Lec. and *L. castaneipennis* Grouv. on stored derris roots, and Miller (1941) records *L. foveicollis* Grouv. on a cocoa pod. *L. alternans* Erichs. was recorded by Mayné (1948), and *L. janeti* Grouv., on several occasions (*e.g.*, Ghesquière, 1922), from tropical Africa. Early records of *L. testaceus* (F.) (Porchinskii, 1913) may refer to *C. ferrugineus*, of which *L. testaceus* Payk. is a synonym. The recent records of *Laemophloeus testaceus* (F.) by Melis (*e.g.*, 1952) must presumably refer to the endemic European species of *Silvanophloeus*, although it seems unlikely that this can be a true storage species.

Summary.

The present world distribution of six species of *Cryptolestes* found in stored produce is delineated from the evidence of specimens collected from a small number of countries and from specimens collected from produce examined in ships at British ports.

It is concluded that *C. ferrugineus* (Steph.) is worldwide, being found in temperate and tropical areas and in humid and dry zones. The range of *C. minutus* (Ol.) is limited by low temperature and low humidity but it is more abundant than *C. ferrugineus* in the wet tropics. *C. pusilloides* (Steel & Howe) is at present confined to the southern hemisphere but may be spreading slowly. It seems unlikely that this species entered stored products handled by the shipping trade much before 1944. *C. turcicus* (Grouv.) has been identified from Europe, North and tropical Africa, Turkey, North America, Uruguay and Argentina, and *C. spartii* (Curt.) only from Europe, North Africa and Turkey. *C. ugandae* Steel & Howe is rare, being found only from Uganda, Nigeria and Ghana.

The type of premises in which each species was found is shown for specimens

collected in Great Britain. *C. ferrugineus* and *C. minutus* are principally found in warehouses and other storage premises, especially on stored cereals and groundnuts. *C. pusilloides* also is principally found in warehouses on stored cereals but shows little sign of becoming an established breeding species. *C. ferrugineus* is found about $2\frac{1}{2}$ times as often as *C. minutus* and about 7 times as often as *C. pusilloides*. On farms and in maltings, *C. ferrugineus* is practically the only species found. *C. turcicus* and *C. spartii* are found mainly in flour mills and other kinds of mill, chiefly in the machinery. In other countries also, wherever the two last-mentioned species have been found, they occupy this same habitat except that, in the U.S.A., *C. turcicus* infests stored grain.

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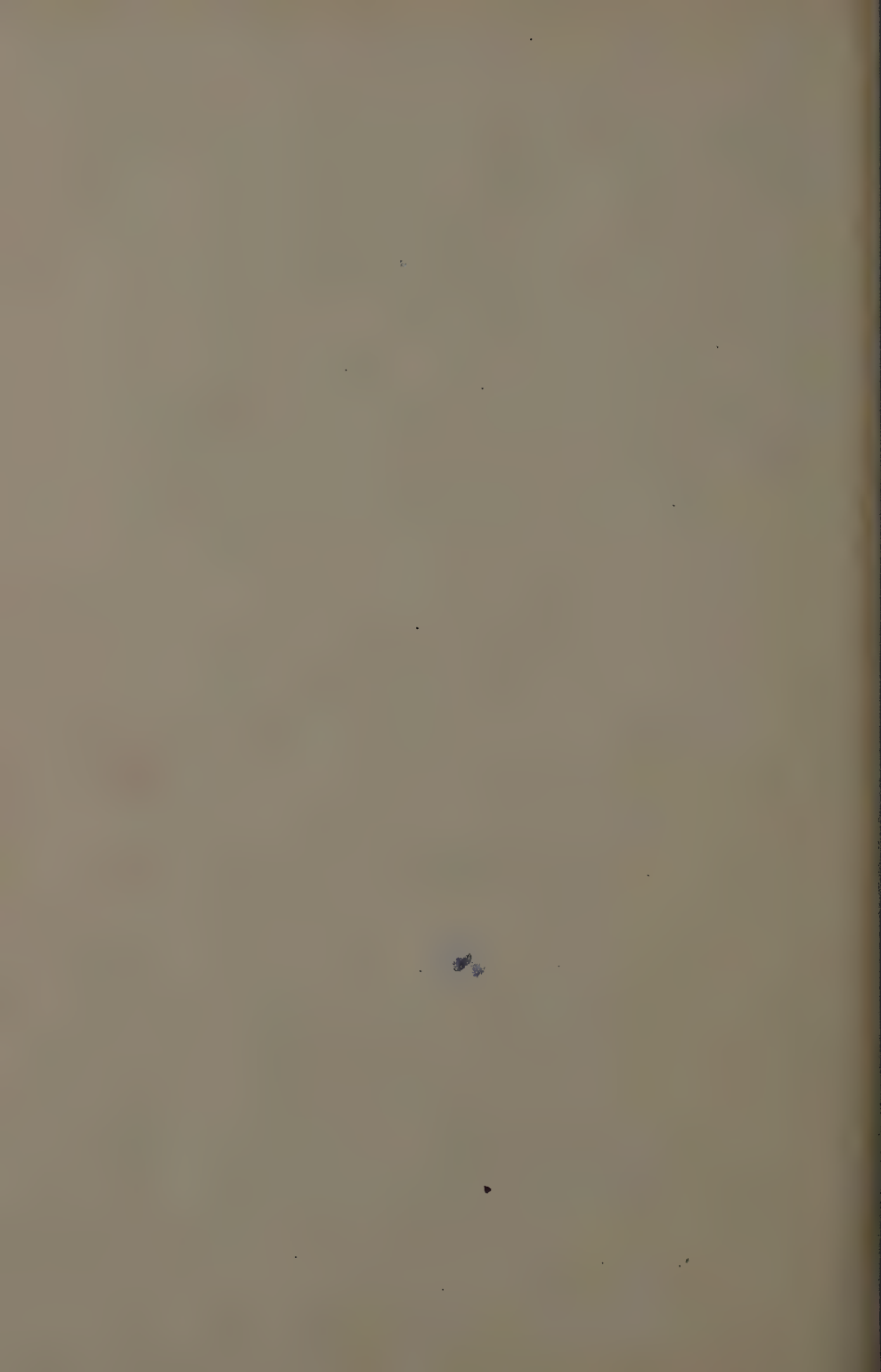
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THE EFFECT OF CURRENT SPEED ON THE DISTRIBUTION
OF THE LARVAE OF THE BLACKFLIES, *SIMULIUM*
VARIEGATUM (MG.) AND *SIMULIUM MONTICOLA*
FRIED. (DIPTERA).

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Phillipson (1956) showed that the tendency of larvae of *Simulium ornatum* Mg. to be confined to lowland streams or watercourses with a low current speed could be explained on the basis of their reactions to current flow, for they are found in waters with a velocity range of 0.5–1.2 m./sec. To what extent current flow limits the occurrence of other species is not known with certainty. The present investigation was undertaken, therefore, to provide information on the reactions

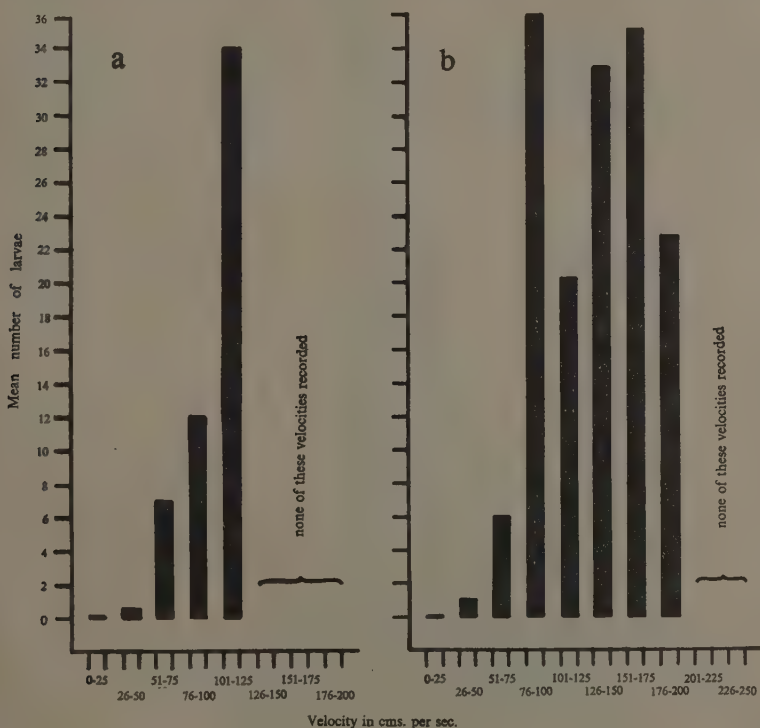


Fig. 1.—Mean numbers of larvae of *S. monticola* occurring at known velocities under natural conditions.

(a) in Troutbeck (based on 1,665 individuals from 125 traps).

(b) in Cross Gill (based on 6,316 individuals from 125 traps).

of larvae of species typical of fast-flowing highland streams. The two species *Simulium variegatum* (Mg.) and *Simulium monticola* Fried. were used in this investigation.

Material and Methods.

Material for laboratory experiments in 1955 and 1956 was obtained from Cross Gill, a tributary of the South Tyne, near Alston, Cumberland. In 1955, laboratory experiments were carried out at the Moor House Nature Reserve, Westmorland, and in 1956 at the Science Laboratories, Durham.

Field experiments were conducted in Cross Gill and in Troutbeck, a tributary of the Tees on the Moor House Nature Reserve, during 1955, but in Cross Gill only during 1956.

Nielsen (1950) suggests that a current velocity of 6 m./sec. is the theoretical upper limit for flowing water (presumably an application of Stoke's Law),

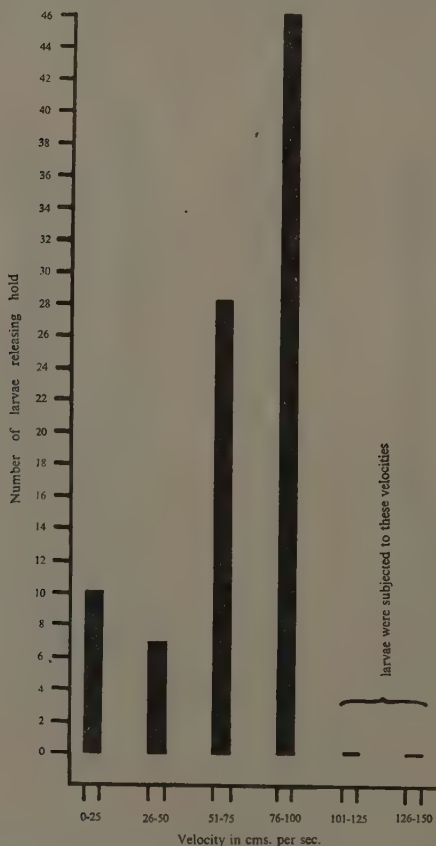


Fig. 2.—Numbers of larvae of *S. monticola* releasing hold at low velocities, plotted against velocity in cm./sec. Based on 91 individuals.

"... but in proper water courses—and even in rushing mountain streams velocities over 3 m./sec. rarely occur". During the present work, current velocities greater than 3 m./sec. were recorded on 13 occasions only, and these in a waterfall during heavy spates.

The methods used by Phillipson (1956) were again employed in the present investigation.

Despite the keys of Edwards (1920), Puri (1925) and Smart (1944), larvae of *S. variegatum* and *S. monticola* could not be separated with any degree of certainty and all identifications were checked by examining pupae produced by various samples of larvae. Of 312 larvae bred and examined in 1955 all proved to be *S. monticola*. During 1956, 224 pupae were reared, 222 proved to be *S. variegatum* and 2 *S. monticola*. It is therefore assumed that all larvae dealt with in 1955 were *S. monticola* and those of 1956 predominantly *S. variegatum*.

Observations on *S. monticola*.

Field experiments.

Experiments in Cross Gill were made from 22nd June to 23rd July 1955 and the results are based on 6,316 larvae from 125 traps.

Between 9th and 15th July, 1,665 larvae from 125 traps were collected in Troutbeck.

The mean number of larvae per trap occurring in each velocity grouping in both Cross Gill and Troutbeck is shown in figs. 1a and 1b. The Troutbeck results indicate that as velocity increased so did the mean number of larvae per trap. The Cross Gill results suggest that the greatest number of larvae occur in the velocity range 0.76–2.0 m./sec. Higher velocities were not obtained because of the prolonged drought of 1955.

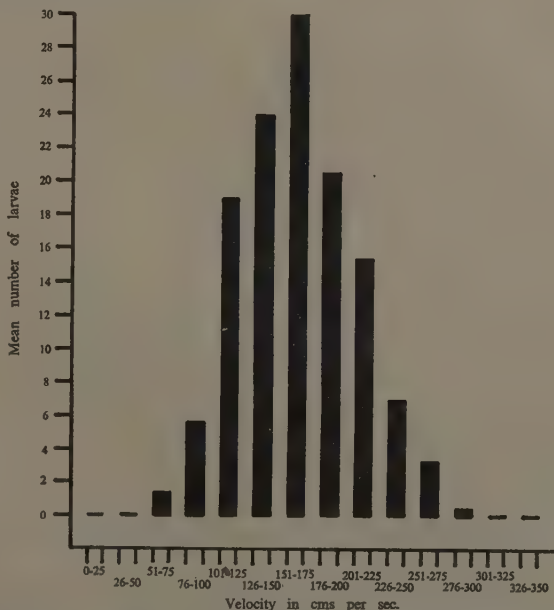


Fig. 3.—Mean numbers of larvae of *S. variegatum* occurring at known velocities under natural conditions. Based on 3,608 individuals from 295 traps.

Laboratory experiments.

The ability of larvae of *S. monticola* to withstand current flow was tested in the laboratory. The current speed was slowly decreased from a speed between 1.25 and 1.5 m./sec. and the velocities taken every 15 min. Each larva was closely watched and the time of release noted. This process was repeated until all larvae had released their hold. Ninety one specimens were subjected to this procedure.

The results (fig. 2) show that larvae released their hold at velocities less than 1.0 m./sec. but retained it at velocities greater than this.

Unfortunately the greatest velocity to which the larvae could be subjected in the Moor House laboratory was 1.5 m./sec., therefore the upper limit of the velocity range preferred by the organisms could not be determined.

Observations on *S. variegatum*.

Field experiments.

All experiments were carried out in Cross Gill during July 1956. The results are based on 3,608 larvae from 295 traps. The mean number of larvae occurring in each velocity grouping is shown in fig. 3, but as the mean is approximately equal to the standard deviation in each group a logarithmic transformation has been used. The transformed curve is shown in fig. 4 and the analysis of variance in Table I.

The results show that the larvae occur in the velocity range 0.51–2.5 m./sec., the greatest abundance being between 1.5 and 1.75 m./sec.

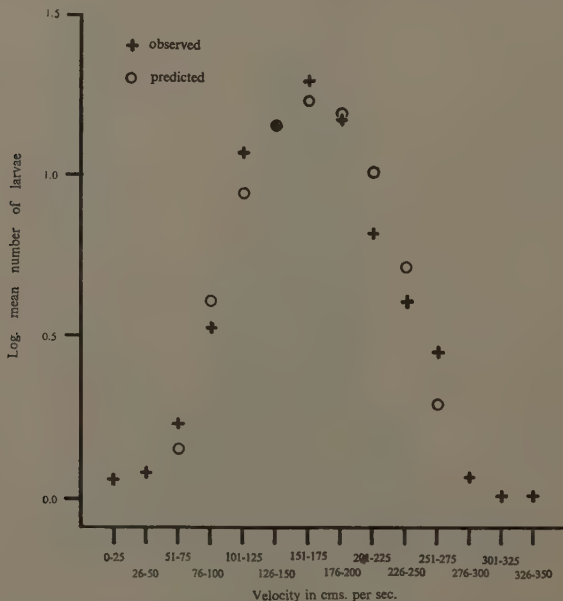


Fig. 4.—Curve of results from fig. 3 after logarithmic transformation.

Laboratory experiments.

In 1956, 38 larvae of *S. variegatum* were subjected to exactly the same procedure as that used for *S. monticola* in 1955.

The results (fig. 5) show that the majority of larvae released their hold at velocities of 0.51–0.75 m./sec. and less but retained it at velocities greater than 1.0 m./sec.

TABLE I.

Analysis of variance of transformed data shown in fig. 4.

	Degrees of freedom	Sum of the squares	Mean square
Regression ..	2	195.5231	97.7616
Residual ..	6	2.3183	0.3864
Within groups	246	61.1517	0.2496
Total ..	254	258.9931	1.0196

In further experiments, 56 larvae, after establishing themselves at velocities of 1.0–1.5 m./sec., were subjected at intervals to increases in velocity, procedure other than change in speed of current was as before.

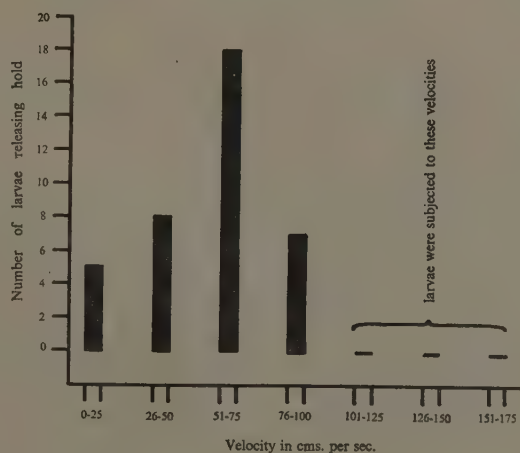


Fig. 5.—Numbers of larvae of *S. variegatum* releasing hold at low velocities, plotted against velocity in cm./sec. Based on 38 individuals.

The majority of larvae detached themselves between velocities of 2.25–3.0 m./sec. but remained attached between velocities of 1.0–2.25 m./sec. (fig. 6). At the highest velocity attainable in the experimental trough (3.5 m./sec.) 6 larvae retained their hold for longer than 15 minutes.

Discussion.

Field and laboratory experiments on larvae of *S. monticola* show that they aggregated in a velocity range of 0.76–2.0 m./sec., very close to the range (0.5–2.5 m./sec.) for *S. variegatum*. Similar figures were obtained by Zahar (1951) in Scotland, whereas in France, Grenier (1949) states that larvae of *S. monticola* and *S. variegatum* were found in velocities of 0.5–0.75 m./sec. but were rare in velocities of 0.95, 0.99 and 1.19 m./sec. In Grenier's (1949) case, on only four occasions are figures for velocities greater than 1 m./sec. recorded, and the maximum current speed given by Zahar (1951) was 96 yds./min. (1.46 m./sec.).

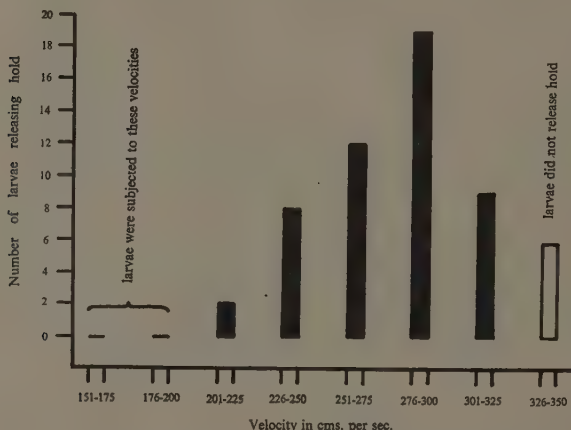


Fig. 6.—Numbers of larvae of *S. variegatum* releasing hold at high velocities, plotted against velocity in cm./sec. Based on 56 individuals.

A list of velocity records for *Simulium* species known to occur in Britain, abstracted and condensed from various authors, is given in Table II. In all cases except those quoted from Wu (1931) and Phillipson (1956 and present paper) the figures indicate velocities in which the species have been found and not the total ranges of velocity in which they could occur. Using the figures given in Table II, a comparison can be made of the velocity requirements of larvae of *S. venustum* Say, *S. ornatum*, *S. variegatum* and *S. monticola*. It can be seen that the velocity ranges of *S. venustum* and *S. ornatum* overlap as do those of *S. ornatum* with those of *S. variegatum* and *S. monticola*. The velocity ranges of *S. monticola* and *S. variegatum* appear to be identical.

From the above figures, *S. venustum*, with a velocity range of 0.17–0.82 m./sec., could be expected to occur occasionally with *S. ornatum* (0.5–1.2 m./sec.), *S. ornatum* with *S. variegatum* (0.5–2.5 m./sec.) and/or *S. monticola* (0.76–2.0 m./sec.), but rarely *S. venustum* with *S. variegatum* and/or *S. monticola*. *S. variegatum* could be expected to occur frequently with *S. monticola*. Species associations found in the field by Zahar (1951) support this. *S. venustum* reported by Grenier (1949) to be found in association with *S. variegatum* has been reidentified by the original author as *S. bezzii* (Corti). (Personal correction to offprint by Grenier (1949).)

The fact that larvae of *S. variegatum* and *S. monticola* can withstand relatively large changes in current speed and can retain their position at high stream

TABLE II.

List of *Simulium* species known to occur in Britain, giving velocities at which these species have been found by various authors. Velocity in all cases is in metres per second. Velocities marked with * are the complete range of current speeds in which the species could be expected to occur, those marked † are the velocity ranges in which the larvae aggregate.

	<i>S. hintipes</i> var. <i>arvernense</i> (Grenier)	<i>S. monticola</i> Fried.	<i>S. variegatum</i> (Mg.)	<i>S. ornatum</i> (Mg.)	<i>S. venustum</i> (Say)	<i>S. tuberosum</i> (Lundst.)	<i>S. reptans</i> (L.)	<i>S. reptans</i> var. <i>galeratum</i> (Edw.)
Edwards (1920) ..	—	—	—	0.54	—	—	—	—
Grenier (1949) ..	1.92	0.5-1.19	0.5-1.19	0.35-0.6	0.5-0.75	—	—	—
Paceud (1942) ..	—	—	—	0.6	—	—	—	—
Phillipson (1956) .. {	—	—	*0.5-2.5 †1.5-1.75	*0.5-1.2 †0.8-0.9	—	—	—	—
Smart (1934) ..	—	—	—	0.32-0.6	—	—	—	—
Wu (1931) .. {	—	—	—	—	*0.17-0.82 †0.3-0.6	—	—	—
Zahar (1951) ..	—	0.37-1.46	0.3-1.46	0.18-1.09	—	0.3-1.46	0.18-1.46	0.18-1.46
Zhivkovit' (1955a)	—	—	—	—	—	—	0.5-0.8	—

	<i>S. equinum</i> (L.)	<i>S. aureum</i> (Fries)	<i>S. nölleii</i> (Fried.)	<i>S. latipes</i> (Mg.)	<i>S. costatum</i> (Fried.)	<i>S. sclopiense</i> (Edw.)	<i>S. sclopiense</i> var. <i>danubiense</i> Zhivkovit'	<i>S. angustitarsis</i> (Lundst.)
Grenier 1949) ..	0.35-0.45	—	0.8	0.4	—	0.35-0.45	—	0.35-0.45
Paceud (1942) ..	—	0.6	—	—	0.6	—	—	—
Zahar (1951) ..	0.24-1.09	0.24-1.09	—	0.18-0.73	—	—	—	—
Zhivkovit' (1955a)	—	—	—	—	—	—	2.0-2.6	—
Zhivkovit' (1955b) ..	—	0.22-0.3	—	—	—	—	—	—

velocities indicates that these species are well adapted to resist the current speeds to which they are subjected during spate conditions.

Another point arising from a comparison of the velocity ranges of the three species is that *S. venustum* and *S. ornatum*, with a relatively narrow tolerance of current speeds, are typically found in watercourses with a moderate current whose velocity does not fluctuate rapidly, whereas *S. variegatum* and *S. monticola*, with a wide tolerance of current speeds, are typical of rapidly flowing hill and mountain streams that are subject to sudden changes in velocity. It should also be noted that a number of larvae of *S. variegatum* retained their hold at velocities between 3.0 and 3.5 m./sec., velocities that are rarely achieved in natural water courses.

Velocity alone could thus be used to explain larval distribution within watercourses, but other factors such as selection of oviposition site by the gravid female, water temperature, character of stream bed, vegetation, food material and competition from other species, no doubt play their part. This is evident from the fact that at the field site in Cross Gill the larval population was wholly of *S. monticola* in 1955 whereas in 1956 it mostly yielded *S. variegatum*.

The different ranges selected by the various species provide a means whereby inter-specific competition will be reduced. On the present data there are no suggestions that a species has the ability to occupy a range vacated by another species with markedly different velocity requirements. In the case of species where velocity requirements are very similar, such as *S. variegatum* and *S. monticola*, the absence of one in large numbers may well allow the other species to enter its range.

Summary.

Field and laboratory studies on the water-velocity preferences of the larvae of *Simulium monticola* Fried. and *S. variegatum* (Mg.) show that both species occur in velocities of 0.5–2.5 m./sec. but aggregate in the velocity range 1.0–2.0 m./sec.

The velocity range that the larvae of different species of *Simulium* can withstand indicates that current speed plays an important part in governing the distribution of blackfly larvae, and perhaps isolating different species along watercourses.

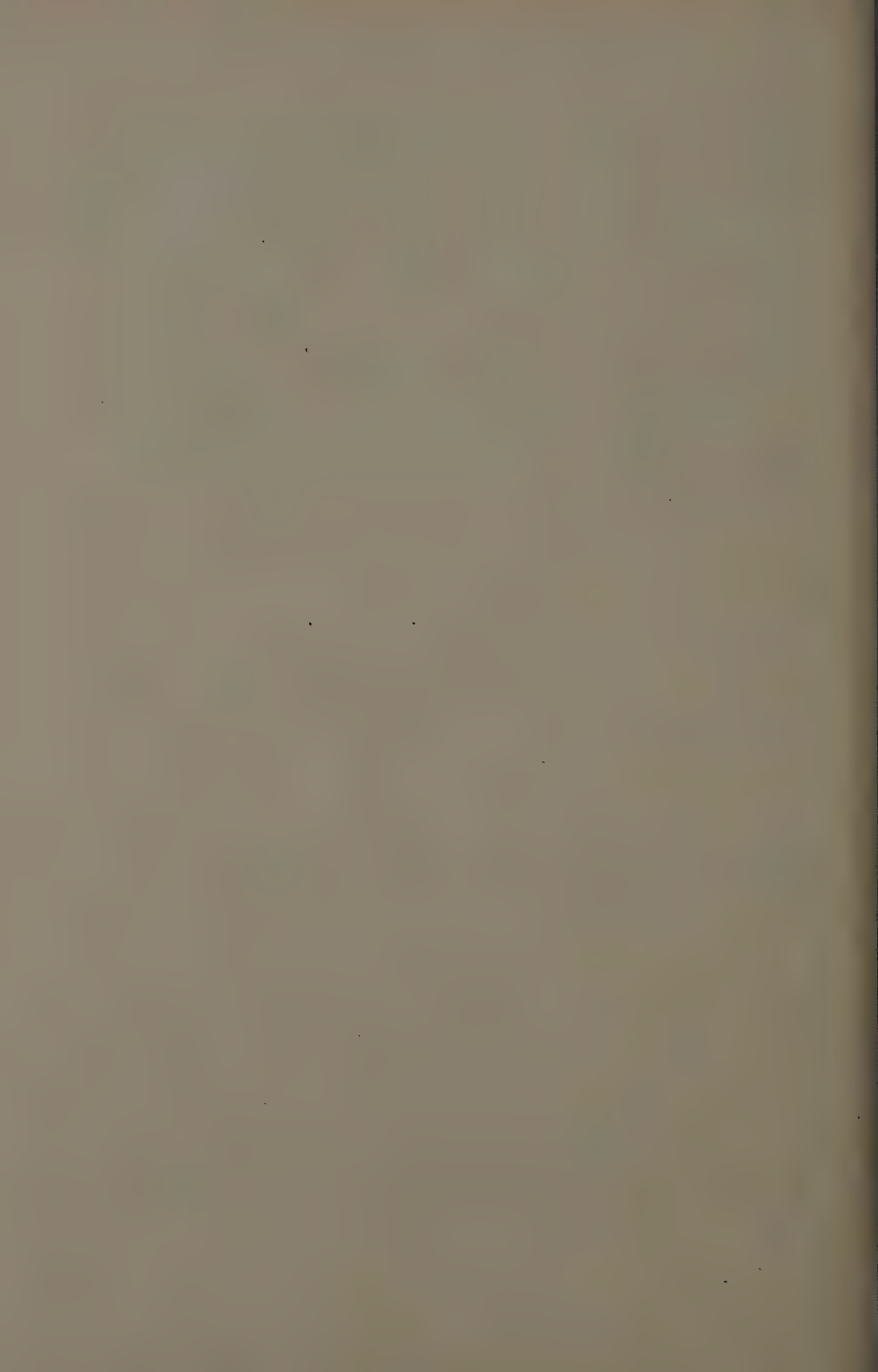
Acknowledgements.

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NOTES ON THE LABORATORY REARING AND BIOLOGY OF THE WHEAT BULB FLY, *LEPTOHYLEMYIA* *COARCTATA* (FALL.).

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References to previous attempts at laboratory rearing of the Wheat Bulb Fly, *Leptohylemyia coarctata* (Fall.), may be found in papers by Ormerod (1889), Petherbridge (1921), Morris (1925), Gemmill (1927) and Gough (1946). The work described in this paper was commenced in order to provide adequate supplies of material for the laboratory testing of insecticides. All stages of the life-cycle were reared in the laboratory, but work was chiefly concentrated on the production of fertile eggs.

Design of Cage for Adult Flies.

Several different types of cage were tried, but it was soon found that small containers constructed from hurricane-lamp glasses were most satisfactory. These were 6 in. high and $4\frac{3}{4}$ in. in diameter, and up to 20 flies were confined in each container. The small numbers in each jar enabled losses due to outbreaks of disease or accidental escape to be reduced to a minimum.

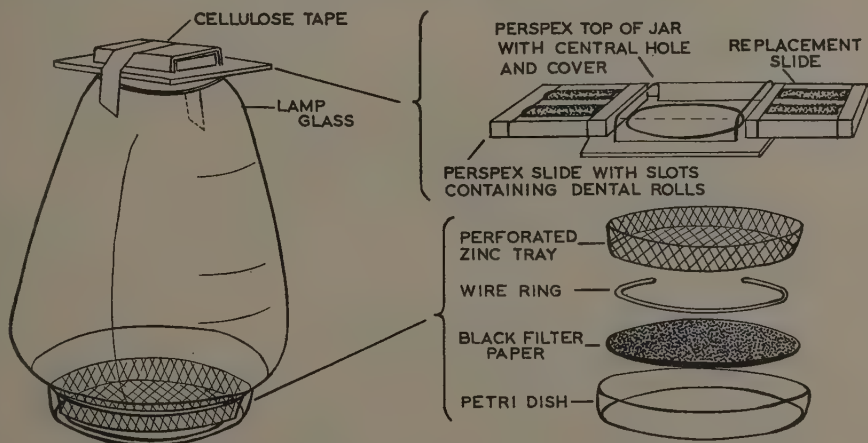


Fig. 1.—Breeding container for Wheat Bulb Fly with details of construction.

In 1953, each cage consisted of a hurricane-lamp glass resting upon the bottom half of a petri dish, the top of the jar being covered with muslin. Liquid food absorbed in cotton-wool was placed on the muslin and the flies laid their eggs in a small amount of soil in the petri dish.

A different rearing container was used in subsequent years (fig. 1), which allowed the eggs to be collected more easily. It also had the advantage that the food could be renewed in less time and did not dry up so quickly. The body consisted of a hurricane-lamp glass as before, but the bottom was sealed by

TABLE I.

Flies kept on different diets, 1953.

Jar	Food	Initial no. of adults		Deaths and escapes before laying commenced	Totals, date before egg-laying commenced		Dates of emergence	Eggs laid	Eggs per female	All dead
		Male	Female		Male	Female				
A	Blood .. Condensed milk .. Honey ..	3	3	0	3	3	16-23.vi.53	539	180	12.x.53
B	Blood .. Condensed milk .. Honey ..	3	3	1 female escaped	3	2	29.vi.53	487	244	30.ix.53
K	Blood .. Condensed milk .. Honey ..	—	3	1 female dead	—	2	7.vii.53	30	15	4.ix.53
C	Bovril .. Honey ..	2	3	2 males dead	—	3	23-30.vi.53	12	4	30.vii.53
D	Bovril .. Condensed milk	3	3	2 males & 1 female dead	1	2	23.vi-1.vii.53	14	7	4.viii.53
J	Blood .. Honey ..	1	3	1 male & 1 female dead	—	2	8-21.vii.53	184	92	16.ix.53
E	Condensed milk Honey ..	2	3	0	2	3	23-30.vi.53	58	19	4.ix.53
F	Honey ..	2	3	0	—	—	23-30.vi.53	0	0	5.viii.53
G	Blood ..	2	3	0	—	—	4-6.vii.53	0	0	27.vii.53

perforated zinc of 2 mm. mesh clipped on the lip of the glass. A wire ring resting on a petri dish bottom containing a sheet of filter paper supported the lamp glass and gave about 1-2 mm. clearance between the perforated zinc and the filter paper. Flies appeared to accept the perforations as a substitute for cracks in the soil and thrust their ovipositors through them, laying their eggs on the filter paper. Black filter papers were used for contrast and the creamy-white eggs could be easily collected without the flies escaping.

The top of the glass was covered by a sheet of perspex, 3 in. square, perforated with a central hole $1\frac{1}{2}$ in. in diameter and held with cellulose tape. Food was absorbed by two dental wicks ($\frac{3}{8}$ in. \times $1\frac{1}{2}$ in.) fitted in a slide that could be moved into position beneath a cover of perspex over the central hole. By pushing with another slide containing fresh food, old food in its slide could be removed without uncovering the central hole and without the escape of flies from the culture. The great majority of flies kept in the laboratory in 1954-56 were confined in this type of jar, at times over 100 being in use.

Diet and Oviposition.

The food of the adults in the field is not definitely known, though they have been observed with extended proboscis on umbelliferous flowers, dead flies, bird droppings and drops of water (Gough, 1946) and we have observed clots of blood adhering to the abdomen.

Eggs have been obtained from flies fed on sugar, pepsin, condensed milk and meat juice (Petherbridge, 1921) and on a diet of cane sugar, glucose, dried milk and meat extract (Gemmill, 1927).

In an attempt to combine features of both the above diets, a preliminary experiment was started in 1953, using flies which emerged from pupae collected in the field.

Four liquid foods were used, both alone and in combination. These were:—

Blood	Uncitrated beef blood mixed with a little water.
Bovril	About 1 cc. mixed in 100 ml. water.
Sweetened condensed milk	1 heaped teaspoonful in 50 ml. tap water.
Honey	1 heaped teaspoonful in 50 ml. tap water.

For mixtures of condensed milk and honey, 1 teaspoonful of each was added to 50 ml. tap water.

In use, 1 ml. of a suspension of the food was pipetted on to the previously damped dental wicks. The foods were renewed every other day. Flies were kept

TABLE II.

Adult diet and oviposition, 1954.

Treatment no.	Diet	Mean eggs per female
1	Honey, condensed milk	5.6
2	Honey, condensed milk and blood	23.1
3	Honey, condensed milk and Marmite	13.6
4	Honey, condensed milk and yeast	12.3
5	Honey, condensed milk, Marmite and yeast	9.5

Least significant difference between means of treatment 2 and any other treatment except 1 (at 5% level) = 17.2.

Least significant difference between means of treatment 2 and treatment 1 (at 5% level) = 18.3.

at 20°C. in a room with 16 hours artificial light per day, at about 60 per cent. relative humidity.

The results of the experiment, summarised in Table I, indicate that egg-production was greatest on a diet of blood, condensed milk and honey, with the exception of Jar K, which contained virgin females only. These results were confirmed in 1954 but in this case Bovril was omitted and instead Marmite (a yeast extract) and bakers' yeast were used, at a rate of about 1 cc. to 100 ml. of water (Table II).

Variance analysis of the untransformed data in Table II shows that the results are significant at the 5 per cent. level.

Treatment (2) (honey, condensed milk & blood) gave significantly more eggs per female than treatments (1) or (5). There was no significant difference at the 5 per cent. level between treatments (2), (3) and (4) or between treatments (1), (3), (4) and (5).

The pupae from which the flies used in the 1954 experiment emerged were

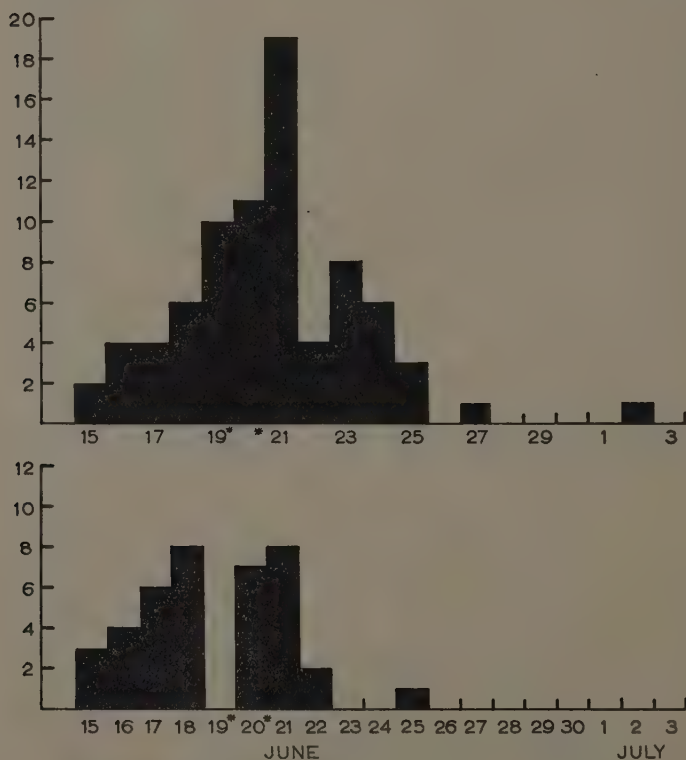


Fig. 2.—Daily emergence of flies (above, females; below, males) from pupae collected in the field and kept at 20°C.

* 10 flies which emerged on each of these days (19th & 20th June) not included as sex not determined.

exposed to a higher temperature than those of the previous year which may account for their reduced fertility.

As the diet of blood, condensed milk and honey proved successful it was used for all the other experimental work described in this paper, except where otherwise stated. Although diet is important for egg-production, flies remained alive for the normal length of time on all the diets tested. The foods used seem to have very little effect on the average expectation of life.

Adults.

Life-span and oviposition period.

It is a common experience when collecting adult flies in the field to find males present about a week before the females begin to appear in any numbers. In the laboratory, however, the peaks for male and female emergence were not so

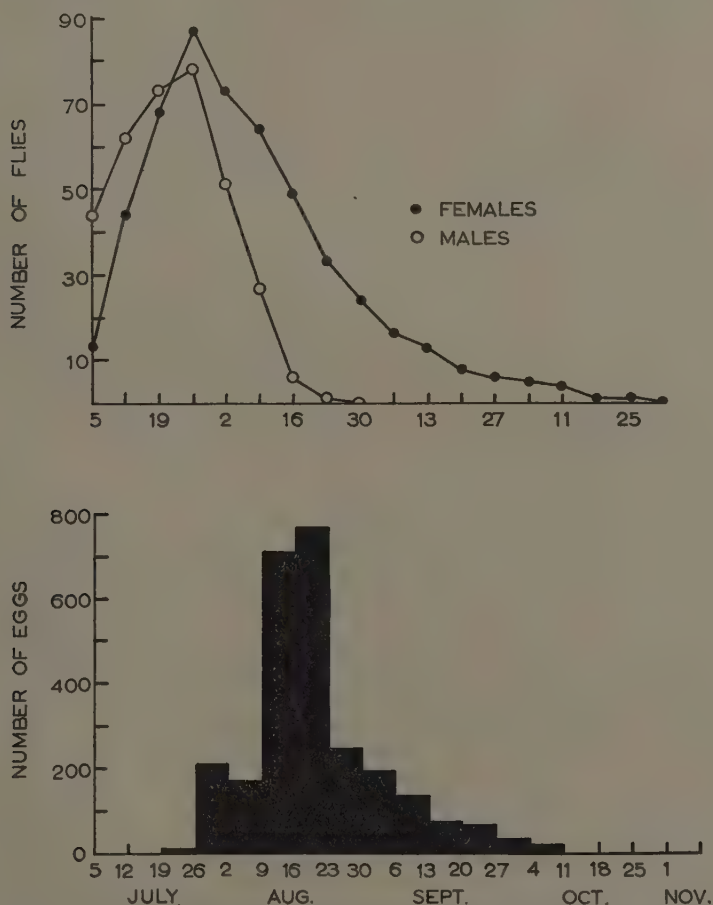


Fig. 3.—Survival of a laboratory population of flies, and number of eggs laid per week.

well separated, as shown in fig. 2. The numbers of each sex which emerged from 251 pupae collected in the field were counted daily. Altogether, 79 females, 39 males and 20 flies whose sex was undetermined emerged, *i.e.*, 55 per cent. The pupae were kept in moist peat or sand at 20°C. Normally the sex ratio is about 50:50 and it is probable that more males than females died during emergence or escaped before being sexed.

In fig. 3, an attempt has been made to show the life-span and egg-laying period of a typical colony of laboratory-bred flies. Infested wheat plants were collected in the field when the larvae were starting to pupate. The plants were placed in large clay pots in an unheated greenhouse and the emerging adults were trapped in tubes.

The graph does not include flies found dead in the traps. The flies were placed in four standard breeding jars, as already described, but with rather a high population per jar (about 50).

The population consisted of 87 females and 78 males. A total of 2,604 eggs were laid, an average of 29.9 per female. The mean length of life of the adults was calculated. This was 26.3 days for the males and 41.6 days for the females.

Eggs were not collected from any jar until at least 12 days after the oldest flies in the culture had emerged. A period of 9–15 days usually elapses between emergence and oviposition. As shown by the graph, the period of maximum oviposition, when about half the eggs were laid, was concentrated in 14 days in mid-August about 3–6 weeks after emergence. A similar egg-laying pattern was also shown by flies captured as adults in the field and brought into the laboratory.

It is also noteworthy that some eggs continued to be laid almost as long as there were living flies in the culture, 15 eggs being discovered at the usual twice-weekly inspection for eggs on 11th October, whilst a female in the same culture-jar survived to between 29th October and 1st November, living for between 96 and 112 days. Batches of eggs laid late in the season are largely sterile.

The exponential shape of the population curve for females shows that in a sufficiently large population a few individuals would be expected to survive for this length of time and in both 1955 and 1954 laboratory-bred flies lived for over 100 days, a fly fed on honey with condensed milk and yeast living for over 145 days. Flies caught as adults in the field have commonly survived to late October or early November.

All the males of the colony had died out by 30th August (fig. 3). Males rarely survive for any length of time, the maximum period recorded being 69–76 days on a diet of honey and condensed milk.

In two instances pairs have been caught *in copula* in the field. One pair caged together failed to produce any eggs, but the female of the other pair, caged without the male, laid eggs within a week after copulation. In the laboratory, copulation can usually be observed within 2–7 days after emergence of the females.

It is of interest that virgin females are capable of laying eggs. Three females that had had no contact with males were caged together and subsequently laid 30 sterile eggs between them (see Table I, Jar K).

Observation shows that flies usually lay several eggs in the same area within a few minutes, thrusting their ovipositors through the perforated zinc until firm contact is made with the filter paper. Eggs are often glued to each other and to the substrate by a water-soluble secretion.

Temperature and oviposition.

This has not yet been investigated in detail because of a shortage of constant-temperature space.

There are indications that a *constant* temperature of 24°C. or above may reduce the number of eggs laid, but flies kept in a *fluctuating* temperature that

often rose to above 30°C. (maximum and minimum not recorded) seemed to lay as many eggs as those kept at a constant temperature of 20°C. Flies in the glass breeding jars were quickly killed by a few minutes' exposure to strong sunshine, an important point if an outdoor or glass insectary is used.

Eggs. •

When kept at laboratory temperature, preliminary development of eggs is rapid, as described by Hedlund (quoted by Rostrup, 1924). No morphological differentiation of the embryo can be seen immediately after the eggs are laid, but at 20°C., 10–12 days after laying, the embryo is apparently fully formed and indistinguishable from a first-instar larva. Heart-beats can be seen, and when the embryo is removed from the embryonic membranes vigorous movements of the cephalic skeleton occur.

Such embryos freed from their surrounding membranes will exhibit a considerable amount of movement (Gemmill, 1927) and will travel for several centimetres, but locomotion is not coordinated as in the normally hatched larvae and it has proved impossible to infest wheat plants with them, even when they were placed in a slit in the stem.

Storage of eggs during diapause period.

M. J. Way (unpublished) has shown that the egg has an obligatory diapause of at least 100 days. Prolonged exposure to temperatures below 12°C. is necessary to break diapause, although preliminary exposure to high temperatures is necessary to complete initial morphological development.

About 15,000 eggs were obtained from insectary cultures in 1954 and 1955 and about 9,000 in 1953. The majority of these eggs were required for insecticide tests. The eggs were kept on moist filter paper in the laboratory until sufficient numbers had accumulated, they were then placed in lots of 500 between two sheets of parachute nylon which were either placed between two layers of earth contained in a piece of glass tubing 2 in. long by 1½ in. diameter or in a petri dish. In either case the containers were then buried in the middle of a 14-in. flower pot filled with potting compost. The flower pots were kept out of doors in a sheltered position and occasionally watered to keep the compost moist.

In late December or early January, the eggs were brought out of storage and examined at a temperature of 5°C. or less. This was because at normal room temperatures the eggs sometimes hatched whilst being examined. Infertile eggs or those containing dead or malformed larvae were rejected. Most of the eggs were then used for insecticide experiments. Those reserved for breeding experiments were stored on damp nylon at 8°C. until they hatched, or stored frozen in ice, as described in the next section.

The chief causes of mortality amongst the eggs were fungal attack and attack by arthropod predators. It was found that eggs kept in the laboratory at various temperatures to simulate outdoor conditions were more subject to fungal attack than those buried outside. Keeping the eggs for long periods on filter paper also encouraged moulds saprophytic on the cellulose, and for this reason nylon was used. To reduce the arthropod populations, some 2 per cent. DDT powder was mixed with soil below the egg containers in the flower pots.

Storage of eggs after normal hatching period.

The very long period of diapause of the eggs of *L. coarctata* is a great hindrance in the study of insecticidal control methods, but it has been found possible to store the eggs at low temperature for long after they would normally have hatched. In late December or early January, eggs are placed between two sheets of nylon or filter paper contained in a petri dish, which is then flooded with water and placed in the ice-box of a refrigerator at -5°C. to -8°C. The

eggs must be surrounded by a film of ice to prevent desiccation. When required, the dishes are brought into the laboratory and left to thaw. Hatching will occur within about 1-5 days. As the number of eggs available for this type of experiment was small, no investigation of the natural mortality of the eggs when stored for different periods of time has been possible so far, but experiments described later in the paper seem to indicate that this is fairly high after several months. However, some eggs still hatch and the larvae will complete development to produce adults when the eggs have been stored for 10-11 months after hatching would normally occur, but all flies obtained from eggs so stored for more than five or six months are sterile.

Water-relations of eggs.

The ability of eggs to withstand desiccation was examined so that correct conditions of storage until hatching could be provided.

Eggs which had been laid the previous day were kept on filter papers in sealed petri dishes maintained at six different humidities: 0, 34, 64, 86, 97 and 100 per cent. R.H., all the dishes being kept at 20°C.

Even at 97 per cent. R.H., half the eggs were flattened and collapsed after 2½ months. At 86 per cent. R.H., all the eggs had reached this condition within 1-2 months, whilst at lower humidities this stage was reached for all the eggs within 1-3 weeks. Older eggs, which had completed morphogenesis, behaved in a similar fashion. Both newly laid eggs and those which had been kept at 20°C. and 100 per cent. R.H. for 20 days collapsed within 6 days when kept at 34 per cent. R.H. Thus the eggs do not appear to possess an effective water-proofing mechanism. However, when the eggs are removed from dry to saturated conditions they can recover from considerable temporary desiccation. Nearly 20 per cent. of a batch of eggs were still alive, or had hatched, 207 days after being exposed to an atmosphere at 34 per cent. R.H. at 20°C. for 13 days, but exposure to these dry conditions for 40 days proved fatal to all the eggs of a similar batch.

Larvae and Pupae.

For the biological testing of insecticides, it is extremely desirable to have all stages in the life-cycle of the insect readily available throughout the year. To do this with an univoltine insect having a long obligatory diapause there are two possible methods. The life-cycle can be shortened by rearing at high temperatures, or slowed down by keeping some stage at low temperatures. In either case a generation will be produced "out of step" with the natural population in the field.

Both these methods have been tried in combination. The hatching of eggs was delayed by low-temperature storage, and when the eggs hatched, larval and pupal development was accelerated by keeping these stages at high temperatures.

Gough (1946) has described a technique of rearing larvae in cut shoots of wheat kept in moist specimen tubes. This was used to determine the length of larval and pupal stages at various constant temperatures (Table III). Adults were only obtained from larvae and pupae kept at 20°C., and at this temperature each stage took from 2½ to 3½ weeks. For comparison, in the field the larval stage lasts 2½-3 months and the pupal stage about 1½ months.

This technique was found to be too laborious for use in rearing large numbers of flies and for most of the work wheat grown in 4-in. or 5-in. flower pots was used. Eggs were inserted in the soil close to the wheat plants.

As this work is still in progress only a summary of the results will be given here.

In all cases, flies only laid eggs when the temperature during the late larval and early pupal period did not exceed 15°C., though younger larvae and older pupae could be kept at 20°C. and still give rise to fertile adults. The numbers of

eggs laid per female were comparable regardless of whether the temperature was raised from 8°C. to 20°C. by increments of 3°-5°C. every 30 days during the combined larval and pupal period or if these stages were kept at 20°C. for 10-21 days and then at 15°C. The time taken for the first adult to emerge was, however, very different, being 93 days and 69 days, respectively.

TABLE III.

Larval and pupal development at various temperatures.

Temperature	No. of larvae tested	No. of pupae obtained	No. of adults obtained	Larval life (days)	Pupal life (days)
8-10°C.	8	1	0	66	—
20°C.	30	11	11	♂ 16-21 ♀ 22-24	♂ 22-24 ♀ 23-27
25°C.	8	1	0	14	—
30°C.	11	0	0	—	—

As would be expected, the time taken to pass through larval and pupal stages varied considerably with the temperature. At 20°C., the first adults were obtained after 40 days, whilst at the same time larvae kept at 8°C. for 80 days and then at 20°C. took 136 days.

Long storage of the eggs before hatching gave rise to a reduced emergence of adults. With eggs hatched in February and early March emergence was up to 36 per cent. Eggs hatched in late June gave up to 17 per cent. emergence, but those which hatched in late November gave only up to 11 per cent. For comparison, a group of eggs placed in potted wheat out of doors in late January gave 30 per cent. emergence.

Long storage seemed also to cause sterility. Regardless of the temperature treatment after hatching, no eggs were laid by flies obtained from eggs hatched later than late June.

It is possible therefore to obtain fertile adults between February and July by storing eggs at temperatures of 0°C. to -8°C. after the completion of diapause. The eggs should be placed amongst potted wheat and kept at 20°C. for 14-21 days, then at temperatures of 11-15°C. for 39-46 days. If then transferred to 20°C., flies will emerge between 60 and 70 days after the removal of the eggs from storage.

Eggs normally hatch in the field in January and early February, the adults appearing in late June, larval and pupal development taking about five months. As fertile adults can be produced earlier in the year than this, as described above, it should be possible to produce young larvae for insecticide experiments several months before they can be found in the field.

Parasites and Predators.

The most common parasite of adult flies appears to be the fungus *Empusa muscae*. This frequently attacked flies brought in from the field to the insectary, and in the field dead flies killed by this parasite can often be seen clinging to stems of wheat.

A peculiar parasitic growth, probably fungal and similar to that described by Smith (1927a & b) was noticed on many flies caught near Harpenden and in the

Fens. This takes the form of a large cyst opening on the ventral surface of the abdomen. Occasionally two cysts occurred in the same fly. Both sexes were attacked, but diseased males were rare. Although the abdominal organs were usually compressed, at least one attacked fly was found with fully developed ovaries. Flies usually died a day or so after the cyst was noticed.

On one occasion dissection of an adult female revealed a large abdominal cyst with no visible external opening. The cyst contained several dozen minute musciform larvae 520 μ long, each enveloped in an embryonic membrane. The preserved material was submitted to Dr. F. van Emden of the Commonwealth Institute of Entomology, who suggested that they might be retained larvae of *L. coarctata*.

The Dung Fly, *Scopeuma* sp., was frequently noticed sucking the juices of *L. coarctata*. This habit has previously been recorded by Dobrovlyanskii (1915) in South Russia.

As noted above, eggs were frequently found to be eaten whilst being stored in diapause. The predators may have been associated Collembola, Staphylinid beetles and mites.

No predators or parasites of larvae were noticed, but an Ichneumonid parasite was bred out of pupae collected in the Fens, and was identified as *Phygadeuon oppositus* Thoms. by Mr. J. F. Perkins of the British Museum (Nat. Hist.).

Summary.

The laboratory rearing of all stages in the life-history of the Wheat Bulb Fly, *Leptohylemyia coarctata* (Fall.), is described. The object of this work was to provide suitable material, in particular eggs and larvae, for insecticide experiments. Details of a container suitable for ovipositing adults are given. Several diets for the adults were compared and it was found that the greatest number of eggs was produced when the flies were fed on beef blood and a mixture of sweetened condensed milk and honey. Females were found to lay up to 244 eggs each; egg-laying commences 9-15 days after emergence and continues almost as long as the females live, but reaches a peak 3-5 weeks after emergence. Some females survived from late June until early November. In a small laboratory population, the average number of eggs laid by each female was 29.9 and mean length of life was 26.3 days for males and 41.6 days for females.

The eggs have a long obligatory diapause period. They have no effective water-proofing mechanism, but can recover from considerable temporary desiccation. A method of storage during diapause is described. The eggs normally hatch in late January or early February, but hatching can be delayed until much later in the year by storage below 0°C.

The effects of temperature on larval and pupal development are described. Larvae will grow at 20°C., but pupating larvae or young pupae are rendered sterile unless kept at temperatures of 15°C. or below. It is shown how a combination of delayed hatching and rapid larval and pupal development can produce fertile flies at unusual times of the year in the laboratory. It is suggested that this may make it possible to produce young larvae in the autumn for insecticide experiments.

In conclusion, some parasites and predators encountered in the field and the laboratory are mentioned.

Acknowledgements.

Our thanks are due to Mr. M. J. Way for many helpful suggestions and criticism throughout the course of this work, to Dr. F. van Emden and Mr. J. F. Perkins for identification of material and also to Mr. A. J. Arnold for his assistance in the design and construction of the breeding jars.

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STUDIES OF CROP LOSS FOLLOWING INSECT ATTACK ON COTTON IN EAST AFRICA.

I.—EXPERIMENTS IN UGANDA AND TANGANYIKA.

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In discussions concerning the determination of the real losses inflicted on crops by insect pests, reference has been made to work in progress in Uganda on the status of the Mirid, *Lygus vosseleri* Popp., and several species of bollworm that attack cotton there (Geering, 1954; McKinlay, 1954). This was one of the main problems to be studied when the Cotton Research Station was opened at Namulonge, Uganda, in 1950. The work with *Lygus* was initiated by Pearson (1952), developed by the present authors, and handed on to their successor, T. H. Coaker, in 1954.

The first part of this study reviews relevant data up to 1952, examines the 1953-54 results in detail, and compares these with results from similar work carried out by one of us in Tanganyika later in 1954. The second part (Coaker, 1957) describes further work in Uganda.

The *Lygus* Problem in Uganda.

In his paper on the biology of *Lygus vosseleri*, Taylor (1945) lists the alternate wild food-plants and suggests that probably the most important of these are certain members of the Leguminosae. Darling (1947) has indicated that a species of *Pseudaerthria*, a wild, perennial, leguminous herb, may be an important host in the elephant-grass zone of Uganda, and that the variation in its times of flowering and maturation may be largely responsible for the variation in the time of the main migration of *Lygus* to the cotton crops in this zone, in which Namulonge lies.

During 1953, counts of nymphs of *L. vosseleri* on selected colonies of *Pseudaerthria* growing in naturally regenerated bush at the Cotton Research Station showed that the rise and fall in the population followed closely the flowering cycle of the host-plant (fig. 1). This year was regarded as a "late" one for *Lygus*, with immigration to cotton continuing to the end of October; it will be seen that the wild host-plant showed a decline in flowering at the end of August, and it had largely matured by the end of October. It was over this period that the main immigration to cotton occurred.

During the same season it was also established that *Lygus* was breeding intensively on black gram (*Phaseolus mungo*) and also on sorghums grown for beer-making. Both these crops usually mature during the break in the rains that occurs between June and August, and this year they were harvested at the end of July. On sorghum, the peak population of *Lygus* adults and nymphs

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was estimated to be 17,000 per acre in July. In 1953, therefore, *Lygus* was being liberated from wild and cultivated host-plants from the end of July to the end of October.

Taylor (*op. cit.*) found that, in the elephant-grass zone, cotton sown in May suffered the heaviest attack from *Lygus*, while cotton sown at later dates incurred a successively decreasing attack. The determination of the optimum date for

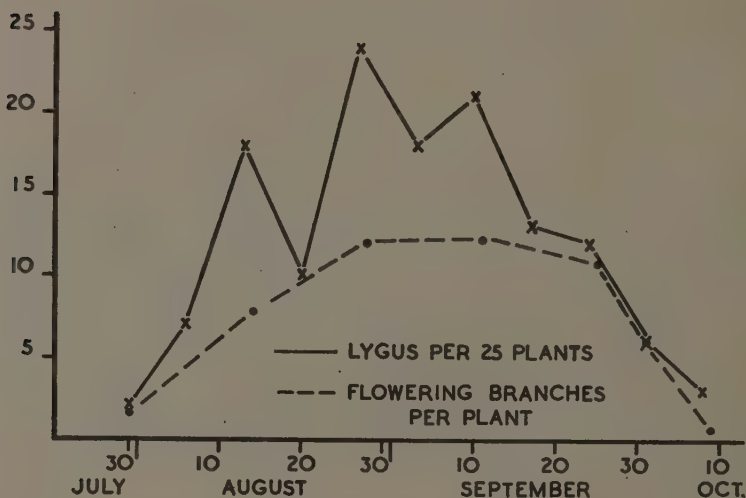


Fig. 1.—Flowering cycle of *Pseudarthria* and associated population of nymphs of *Lygus vosseleti*, Namulonge, 1953.

sowing cotton in this zone is therefore intimately connected with the *Lygus* problem, and the position has been well summarised by Manning (1949). Referring to a sowing-date trial conducted at the Agricultural Research Station, Kawanda, in 1946-47, he states: "Previous trials conducted over a number of years indicated that May-sown cotton gave the highest yields. The validity of these results was questionable since contiguous plots were used for the different planting dates; insect migration could have taken place from early to later sown cotton. A new type of sowing-date experiment (designed to minimise the effect of this inter-plot effect), indicated that there was no evidence that insect interference contributed to the lower yields of cotton sown in August and September. Rather, the cotton sown in May and June had the highest *Lygus* leaf-damage counts. The optimum sowing date for this trial was May 1st, but yield decline was not demonstrable before June 26th."

The evidence from this trial, and other independent trials conducted over a number of seasons, has shown that in Uganda the early part of June is the optimum period for sowing cotton near the equator, in spite of the heavier *Lygus* attack suffered under such conditions. This is well illustrated in fig. 2 of Manning's paper. It follows that experiments aimed at determining the maximum loss of yield resulting from *Lygus* attack under these circumstances should be conducted on cotton sown in early June.

Experiments at the Cotton Research Station, Namulonge, Uganda.

The difficulties encountered in protecting plots of cotton from *Lygus* attack by means of insecticides have already been briefly reported by Pearson, Geering & McKinlay (1952), McKinlay (1953) and Geering (1954). These led to the development by McKinlay of the technique of spraying single rows of cotton at frequent intervals with formulations of DDT. Cotton treated in this way effectively repels *Lygus* adults, and thus, from treated single-row lengths randomised in a large area of untreated cotton, it is possible to determine the yield of cotton when it is not attacked by *Lygus*. The insecticidal treatment also gives effective control of bollworm larvae.

The principal pests of cotton at Namulonge, in their seasonal order of occurrence, are *L. vosseleri*, the bollworm (*Heliothis armigera* (Hb.)) and spiny bollworms (*Earias* spp.). Bollworms had not been found in large numbers on cotton at Namulonge, but the results in 1952 suggested that a comparatively light attack by these insects in the latter part of the season could cause a greater reduction of yield than an earlier and apparently heavy attack by *Lygus* (Geering & McKinlay, 1953). The peak periods of attack in 1952 were August–September and October–November, in the case of *Lygus* and bollworms, respectively.

Methods.

It was decided to study the effects of the two types of pest by protecting cotton from each of them separately and both together, by the method of the single sprayed row. An experiment was accordingly carried out in the 1953–54 season in which there were three main treatments, namely, spraying during August–September (a), October–November (b), and August–November (c), together with unsprayed controls (d). The treatments were arranged in a 4 × 4 Latin square. In addition, and immediately adjacent, a second experiment having a similar layout was performed in which the spray periods were September and October (e), October (f) and September–November (g), with unsprayed controls (h). The object of the second experiment was to see whether it was necessary to apply control during the whole period of attack of the two types of pest, again either separately or together.

These periods applied to the June sowings, and it is necessary to digress on the subject of sowing date. Manning's results referred to successions of sowings on bare fallows, with no crops except weeds, during the early part of the rains. Cotton sown in early June in Buganda must necessarily occur either on fallow, or on freshly broken land. It is, however, a common practice in the elephant-grass zone for maize to be grown during the first rains (March–June) and cotton to be sown after the maize is harvested; with indigenous varieties of maize, this means sowing the cotton in August. It will be seen from Manning's graph that, even in the absence of a previous maize crop, this will result in a large drop in yield.

To test the effect of controlling insects on both early and late-sown cotton, therefore, the single sprayed rows were incorporated into an experiment designed by H. L. Manning to test annual yield levels on what amounted to a single and a double cropping system, that is, on a crop of cotton alone sown in early June, and on one following a maize crop, and therefore sown in August. These are referred to as "early" and "late" sowings.

The treatments applied to the late-sown cotton covered periods similar to those on the early sowing in the sense that they aimed at providing protection from the same two pests during the periods that the cotton was susceptible to them. The whole spraying period was thus necessarily later, and did not commence until mid-September when *Lygus* activity was declining. Treatments i–l were the late equivalents of a–d, and m–p aimed at observing the effect of very limited control of *Lygus* and bollworms.

All treatments on both sowings are listed below.

Experiment Treatment		Period of protection
Early-sown cotton (June 15th)		
1	<i>a</i>	August and September
	<i>b</i>	October and November
	<i>c</i>	August, September, October and November
	<i>d</i>	Nil (control)
2	<i>e</i>	September and October
	<i>f</i>	October
	<i>g</i>	September, October and November
	<i>h</i>	Nil (control)
Late-sown cotton (August 10th)		
3	<i>i</i>	Last half September and October
	<i>j</i>	November and first half December
	<i>k</i>	September, October and November
	<i>l</i>	Nil (control)
4	<i>m</i>	Last half September
	<i>n</i>	October
	<i>o</i>	November
	<i>p</i>	Nil (control)

The layout of each Latin square and the spacing of the sprayed rows are illustrated in fig. 2, where treatments *a-d* are given as an example.

The experiments were fitted into the standard contour strips in which the station is laid out. The layout of each Latin square thus shows the single, sprayed rows, each 25 ft. long, arranged across these strips, in which the rows are spaced at 3 feet. Between each sprayed row there were 7 unsprayed rows. In order to reduce interference between treatments, the position of the sprayed rows was staggered in the manner illustrated. Each experiment was repeated on



Fig. 2.—Latin-square arrangement of single-row treatments, *a-d*.

three very widely separated areas on the Station,* thus giving a total of 12 replications of each treatment, four in each area. During the periods of protection, a spray containing 0.2 per cent. DDT, made up from a paste formulation containing 50 per cent. DDT, was applied weekly to run-off to the treated plants. There was no evidence that these sprays had any direct phytotoxic effect upon the cotton plants.

Yields.

The yields from the various treatments, in pounds of seed cotton per acre, are given in Table I.

There was considerable variation in yield between localities and consequently when treatment means over all localities were considered, only two treatments, *n* and *o*, both on the late-sown cotton, showed significant increases in yield over controls. Analysis within localities, however, showed that in one of them (Nalumuli) treatment *k* gave an increase of 82.5 per cent. over control.

Harvesting is normally completed in 2-3 pickings at 3-4 week intervals, but in order to follow the manner of formation of the crop in this experiment, sample pickings were taken at weekly intervals. On the early sowing, this necessitated a total of thirteen pickings. These were then bulked into four consecutive groups, and the successive yields from the four treatments, *a-d*, constituting Experiment 1, are shown in Table II.

TABLE II.

Yields in Expt. 1, divided into four consecutive picking periods.

Weekly pickings	Total yield over all areas (kg.)				
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	
1-3	6.88**	4.03	5.88**	2.88	
4-6	7.24	9.91*	8.41*	7.12	
7-9	3.18	5.92*	4.54	5.57*	
10-13	1.58	1.37	0.85	3.39***	
Total	18.88	21.23	19.68	18.96	No significant differences

In each group of pickings, yields marked by asterisks differ from those without at the following levels of significance: *** $P = .001$, ** $P = .01$, * $P = .05$.

It will be seen that, in the first three pickings, treatments *a* and *c* significantly outyielded treatments *b* and *d*. The accent changes throughout the picking periods, until in the last the controls (*d*) gave a very significantly greater amount of cotton than any of the protective treatments. The patterns of crop formation are further illustrated in figs. 3 and 4, where the cumulative yields have been plotted. In the picking curves for the sprayed early-sown cotton (fig. 3), only treatments *c* and *g* have been included, and these show that the potential yields and the rates of crop production differ in the three areas in which the experiments were replicated. They also show that after the maximum period of protection (*c*), the resulting crop was produced earlier, but was no greater than the controls; and in fact in one locality it was a shorter spray period (*g*), which produced the greatest increase in yield (35%).

* Designated Nalumuli, Kirimantungo and Sendusu.

In fig. 4, selected picking curves from the late-sown experiments are given to show comparisons between controls (l) and complete protection (k) in Expt. 3, and between controls and the best treatment in terms of yield in Expt. 3 and (Sendusu only) Expt. 4. Here it may be noted that in the late-sown cotton all harvesting was completed in seven weeks, and that, in Expt. 3, only at Nalumuli

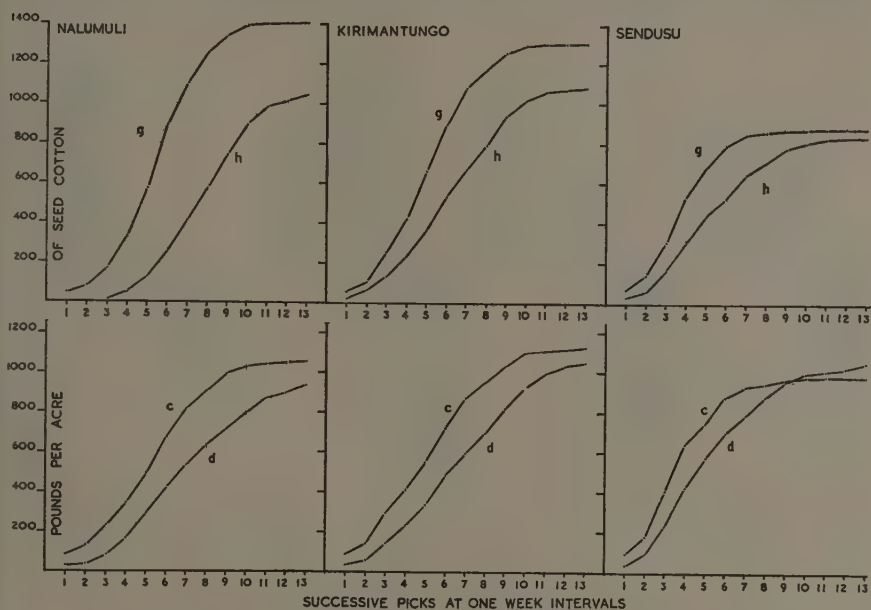


Fig. 3.—Early-sown cotton. Cumulative yields from single sprayed rows, Cotton Research Station, 1953-54. *d*, *h*, controls; *c*, 17 weekly sprays (Aug.-Nov.); *g*, 13 weekly sprays (Sept.-Nov.).

was complete protection the best treatment; in the other two cases the best treatment was the shorter, late-season period of protection (*j*). Again, however, the spray treatments yielded their crops earlier, whether or not these were significantly greater than the controls.

Throughout the season, records were kept of the amount of visible leaf damage by *Lygus*, and the populations of bollworm larvae. There was no correlation between these counts and final yield on the early sowing, but on the late sowing there was a significant negative correlation between yield and counts of bollworm. This aspect of the work will, however, be dealt with in Part II of this paper (Coaker, 1957).

Development of the crop.

Detailed records of the developing crop were taken at monthly intervals on all treatments. This involved recording the situation at each fruiting point, *i.e.*, whether a flower bud or boll was present, or had been shed. Discussion of these fruiting-point counts will be limited to treatments *a-d* (Expt. 1).

The histograms in fig. 5 show the net production of flower buds* during each month on 60 plants of each treatment (20 from each area). The net production

* The number of buds formed, minus the numbers converted to flowers or shed as buds.

of buds during November was of course negative, since formation of new buds had almost ceased whilst those present in the previous month were being lost by conversion into flowers or were being shed. It will be seen that protection from insects had a very marked effect upon the number of buds that were retained at any given time, and this must be responsible for the differences already noted in

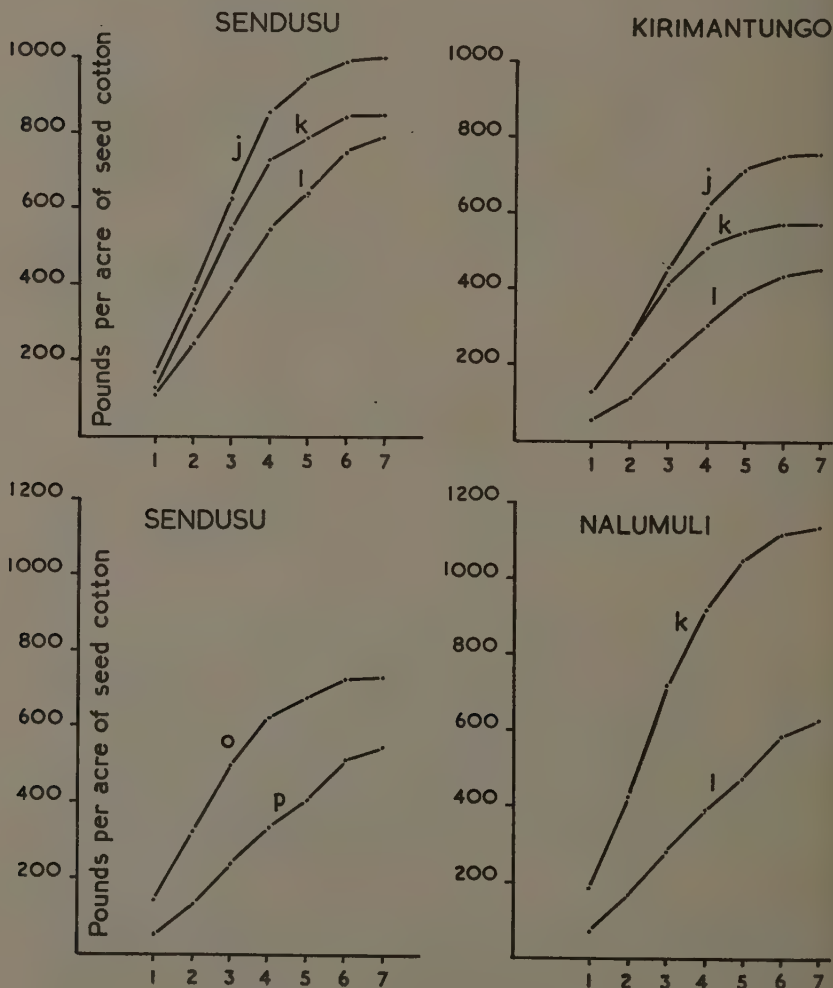


Fig. 4.—Late-sown cotton. Cumulative yields from single sprayed rows, Cotton Research Station, 1953-54. *l, p*, controls; *j*, 5 weekly sprays (Nov.—early Dec.); *k*, 11 weekly sprays (mid-Sept.—early Dec.); *o*, 4 weekly sprays (Nov.).

the time at which the crop matured. It was also noticed that there was a very rapid decrease in the number of buds present once the plants began to ripen a crop of green bolls. Table III shows the number of buds present on 26.xi.1953, shortly before picking started. Treatment *d*, the control, which lost more buds during the early part of the season than did the treated plants and therefore

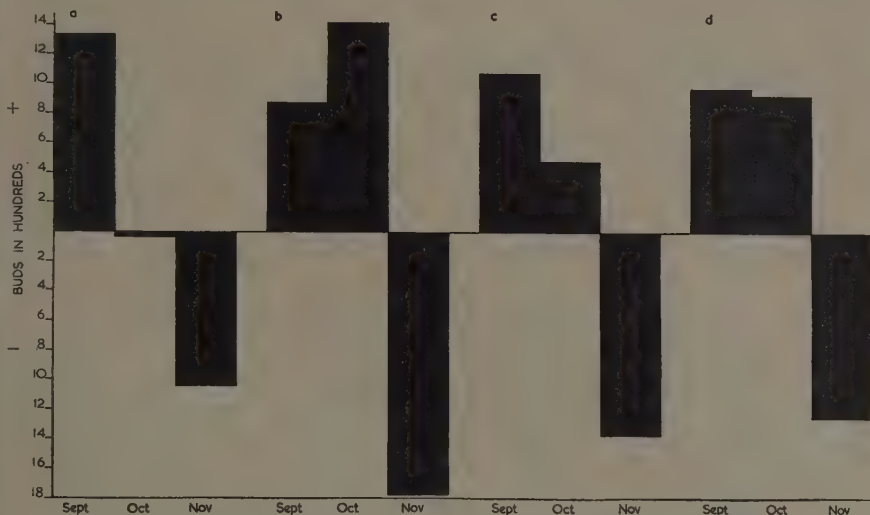


Fig. 5.—Total buds formed and retained during Sept.-Nov. The counts were made on 20 plants per treatment in each locality. (For explanation of treatments (*a-d*), see text.)

cropped later, had very significantly more buds present at this time than had the other treatments, whereas *a* and *c*, which had both been protected early in the season, had the lowest number of buds.

Further evidence of the effect upon bud retention of bearing green bolls is afforded by a count of what Farbrother (1953) has called "match-head" shedding.

TABLE III.

Number of flower buds present on Expt. 1 on 26.xi.53.

Locality	Treatments				Total
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	
Nalumuli	213	260	130	327	930
Sendusu	1	96	16	153	266
Kirimantungu	184	269	132	407	992
Total	398	625	278	887	2188

Counts made on 20 plants per treatment per locality.
Significant differences: $d > a, b, c$ ($P = .001$); $b > a, c$ ($P = .01$).

This term describes the tendency of small buds, less than $\frac{1}{4}$ th inch long, to turn brown and be shed for no reason that can be directly attributed to physical damage by insect pests. Table IV shows the number of sympodia on which the small visible terminal flower bud was present, or absent, on 20 plants per treatment in the Sendusu area on 7.xi.1953.

TABLE IV.

Number of terminal flower buds present or missing on Expt. 1 on 7.xi.53.

		Treatments			
		<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
Buds present (P)	..	201	365	171	423
Buds absent (A)	..	481	406	258	310
P/A	0.42	0.90	0.66	1.36

Counts made on 20 plants per treatment per locality.

Since match-head shedding was greater on those treatments that were forming the earliest crops, the implication is that the ripening of a crop can result directly in a loss of very young flower buds and, as an extension, that within the limits of the environment there may be a ceiling to the crop that a cotton plant can carry, and that once this is reached, yield is checked by the shedding of flower

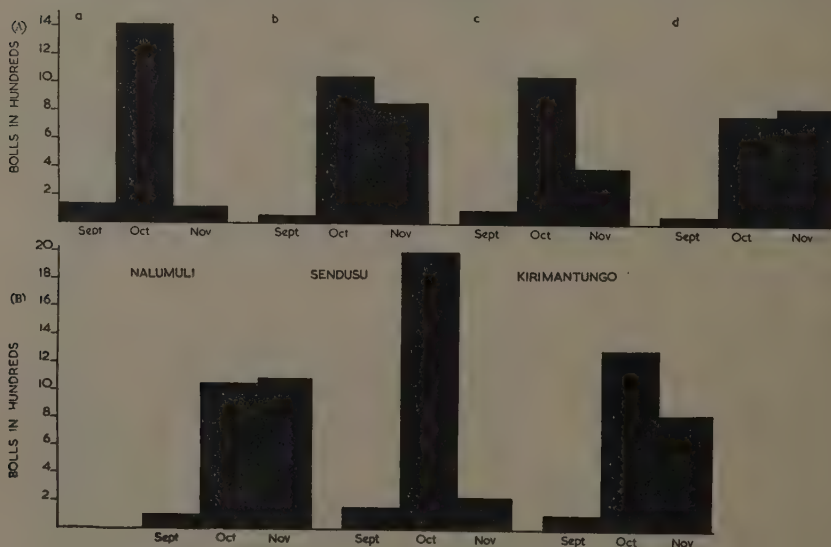


Fig. 6.—Total bolls formed and retained during Sept.-Nov.
 (A) 20 plants per treatment, 8 areas bulked.
 (B) Counts on 60 plants per area for the continuously sprayed treatment (c).
 (For explanation of treatments (a-d), see text.)

buds. There was a similar effect shown in the physiological shedding of very young green bolls.

Fig. 6 shows the number of green bolls formed and retained during September, October and November, (A) for each treatment, bulked over the three areas, and (B) for the continuously sprayed treatment, *c*, in each area separately. Once again it can be seen that, although there were no differences in total yield, protection from insect attack had a very marked effect upon the time at which the crop was formed.

The chief points demonstrated by this experiment at Namulonge were:—

1. The cotton plant is able to sustain considerable insect damage to its fruiting bodies* and yet "recover" so that the total yield is unaffected, particularly where the growing season is long.
2. Because of this ability of the cotton plant to recover, it is difficult to assess the point at which insect damage will result in a loss of yield and make insecticidal protection profitable.

Experiments at Ilonga Research Station, Eastern Province, Tanganyika.

It seemed that any increase in the severity of the insect attack, or any shortening of the season, due to lack of rain, would make a considerable difference to the plant's reaction to insect attack. To investigate this aspect, the work was continued at Ilonga, in the Eastern Province of Tanganyika, and the results from two trials are given which supplement the Namulonge work.

The background to cotton growing in this part of Tanganyika, including the insect-pest situation, has been described by Bebbington & McKinstry (1945). Briefly, there is only one rainy season, extending from December to May, the remaining six months being almost without rain, and cotton is normally sown in March. The cotton crop is usually subject to severe attack from the bollworm, *Heliothis armigera*, the main incidence of which is in June.

Trial 1.

In this trial, in order to have the longest possible growing season, a small plot of cotton was sown on 31st January 1954, and sprayed weekly to run-off with 0.2 per cent. toxaphene as a protection against loss of fruiting bodies as a result of attack by insects, mainly *Heliothis*. The following "treatments" were then effected, consisting of the removal of all flower buds, at weekly intervals, for various periods (in weeks) dating from the time of appearance of the first flower buds: A, 1; B, 2; C, 4; D, 8; E, 12; and F, nil (no buds removed). There were only five single-plant replications of each treatment and these were laid out in randomised blocks. In order to avoid any differences due to shading effects, each treated plant was completely surrounded by untreated plants.

Although detailed records were taken of crop development, the main results of the trial are shown equally well by Table V, which gives the amount of cotton picked weekly off each treatment.

Analysis showed that because of the high plant-to-plant variation and the small number of plants used, there were no significant differences between the treatments as regards total yield. When it is remembered that one of the treatments had all flower buds removed for twelve weeks and nevertheless gave a yield not significantly different from that of the undamaged controls, the results are probably more striking than the recovery of the unprotected plants at Namulonge. These results are also similar to those obtained at Namulonge in that although the treatments showed no significant differences in total yield, there was a very marked difference in the time and rate at which the crop was

* The term "fruiting body" is used to cover both flower buds and bolls.

TABLE V.

Time of production of crop from cotton protected by insecticides, following disbudding for varying periods, Ilonga, Tanganyika, 1954.
Yield (seed cotton) at successive weekly pickings (totals from 5 plants per treatment).

Treat- ment	Length of disbudding period (in weeks)	1.vi	7.vi	14.vi	21.vi	28.vi	5.vii	12.vii	19.vii	26.vii	3.viii	9.viii	16.viii	23.viii	30.viii	6.ix	12.ix	Total
A	1	0	10	93	123	151	176	82	53	21	4	0	0	11	0	1	0	725
B	2	0	0	6	86	155	228	124	115	94	74	11	29	2	0	0	0	924
C	4	0	0	0	0	0	0	289	279	214	108	79	21	3	0	0	0	993
D	8	0	0	0	0	0	0	0	0	9	193	345	343	131	45	45	2	1113
E	12	0	0	0	0	0	0	0	0	0	0	0	26	98	608	317	104	1153
F	0	80	124	166	143	189	173	128	88	59	37	11	11	2	0	0	0	1211

For explanation of treatments, see text (p. 843). Cotton sown 31.i.54.
There were no significant differences between treatments as regards total yield.

picked. It can be seen from Table V that the crop from the untreated control* (F) was gathered in 13 weekly picks, and never exceeded 200 g. from five plants in any one week, whilst the treatment in which all flower buds were removed for 12 successive weeks (E), gave its entire crop in five weekly picks, of which the largest exceeded 600 g.

The experiment thus confirmed the marked ability of the cotton plant to recover from the damage to flower buds caused by an insect attack, as represented by an equivalent removal of fruiting bodies, provided that the growing season is sufficiently long.

Trial 2.

The effects of an intense attack by insects occurring during a short season is shown by a formal insecticide trial carried out on late-sown cotton at Ilonga in 1954.

This trial was a comparison of four different insecticidal treatments for the control of *H. armigera*, and comprised eight replications of each treatment, each of $\frac{1}{8}$ acre, arranged in a randomised-block layout.

The treatments were as follows:—

- A. 10% DDT + 3% γ -BHC dust.
- B. 20% toxaphene dust.
- C. 10% DDT + 10% toxaphene dust.
- D. 0.8% DDT + 0.25% γ -BHC as an emulsified solution.
- E. Control (no treatment).

All dusts were applied at 10 lb. per acre and the emulsified solution as a low-volume spray at 12 gal. per acre.

The yields (in lb. seed cotton per acre) were as follows: A, 968; B, 834; C, 777; D, 973; and E, 211. A–D were significantly better than E ($P = 0.001$), both A and D were significantly better than either B or C ($P = 0.01$), but neither A and D nor B and C differed significantly.

The main interest in this trial lies in its demonstration of the very heavy reduction in yield that can occur when bollworm attack is not controlled. This cotton was sown comparatively late (23rd March) and whilst it still gave a reasonable yield where bollworm damage was controlled, it had not the powers of recovery of the early-sown cotton (31st January), in which even the removal of all flower buds for 12 successive weeks did not significantly reduce yield.

Discussion.

Effects similar to those described here, following the use of insecticides on cotton, have been recorded in the United States of America. Hanna & Mistic (1953) showed from their own work and that of others that early-season protection of cotton resulted in earlier yielding but no increase in total size of crop. Hanna & Gaines (1952) also reported experiments showing that insect control on cotton can sometimes give rise to earlier yields but no increase in crop size. Gaines & Wipprecht (1950) suggested that protection from insect attack early in the season may force the plant to fruit early and hence even reduce yield. Dunnam, Clark & Calhoun (1943) investigated the effect on the cotton plant of removing squares (*i.e.*, flower buds) and concluded that if rainfall was below normal, any loss of fruiting bodies reduced yield, but if rain was above normal, and the growing season therefore longer, removal of buds for up to six weeks had no effect upon yield. Earlier work by Eaton (1931) on the effect of disbudding, demonstrated actual increases in yield resulting from this artificial loss of early-formed fruiting bodies.

* *i.e.*, plants with no buds removed, but protected from insect attack.

It has already been concluded by Pearson & Mitchell (1945), and the earlier investigators to whom they referred, that owing to its structure and manner of growth the cotton plant produces many more flower buds than it can nourish. This is largely due to the fact that on sympodial branches, each leaf is accompanied by a flower bud, but that this flower bud requires more than one leaf to supply the nourishment needed to form a mature boll. Thus it is found that, even in the absence of insect attack, a very great proportion of potential bolls are shed as buds, or immature bolls. It would then seem reasonable to assume that to a certain extent loss of fruiting bodies following damage by insects is unimportant, for the plant would shed them in any case from "physiological" causes. With a long growing season, the net effect of early loss of buds from insect attack is to ensure that the plant makes its crop from the later-formed fruiting points that in normal circumstances would have been shed from physiological causes. It was apparent from the Namulonge work that once the plants began to ripen a crop of green bolls, there was an almost immediate increase in the rate of bud shedding. Similar results were reported by Pearson & Mitchell (*op. cit.*) from Nyasaland, where they were considering the effect on cotton of attack by red bollworm (*Diparopsis castanea* Hmps.). Farbrother (1953, 1954), however, has been demonstrating at Namulonge that internal factors, such as the weight of crop being carried, do not alone control the pattern of flowering and subsequent crop retention.

It would thus appear that there is a limit to the amount of crop that one plant can carry at any given time, and that, once this has been reached, any excess fruiting bodies are shed, so that, broadly, the effect of insect attack is only to delay the time at which the balance is reached. It is here that a possible explanation can be given for those cases in which early insect damage has shown a tendency to increase yield. The results obtained by Farbrother (1954) support the above theory that the size of the crop borne by a plant is determined by the leaf area, so that the longer crop formation is delayed, the larger will be the available leaf area and the greater the number of bolls that the plant can eventually nourish.

However, the cotton plant's powers of recovery are not unlimited, and when the season is short or the insect attack prolonged and severe, the point at which recovery is still possible is passed and the yield consequently reduced. This is particularly well demonstrated by the second of the two trials carried out at Ilonga.

These effects all have a very important bearing on the use of insecticides for the control of cotton pests. The fact that attacks by insects are observed on cotton plants is no indication that their control by insecticides would always give a profitable increase in yield. Whilst feeding by insects on cotton is in one sense "insect damage", it has to be remembered that it is only important when it actually reduces the yield. It follows that before insecticides are used to control a cotton pest, an attempt must be made to assess the point at which, under local conditions, further damage would cause reduction of yield. Only from that point onwards would the use of insecticides have any chance of being profitable.

Summary.

The loss of crop following insect attack on cotton was studied in 1950-54 at the Cotton Research Station, Namulonge, which is situated in the elephant-grass zone of Uganda, in which the principal pest of cotton was originally considered to be *Lygus vosseleri* Popp. In 1953, the main invasion of cotton by *Lygus* occurred during September and October, originating from cultivated crops of black gram (*Phaseolus mungo*) and sorghum and from the wild perennial legume, *Pseudarthria* sp., which earlier workers had considered important. Trials in this zone have demonstrated, over a number of years, that early June is the optimum date for

sowing cotton, although such sowings receive the heaviest attack by *L. vosseleri*. Experiments to determine the maximum loss of yield due to *Lygus* should therefore be made with cotton sown at this time. Bollworms are also important pests, notably *Heliothis armigera* (Hb.) and *Earias* spp., their attacks following those of *Lygus* and being heaviest in October–November.

An experimental procedure was devised to evaluate the effects of the two principal types of pest. This depended upon the fact that single rows of cotton treated at weekly intervals with an appropriate insecticide and sited among larger areas of untreated cotton are protected against attack by *Lygus*, the adults of which are mobile and are repelled, and by bollworms of various species, which are killed. The two types of pest differ in their time of incidence, that of *Lygus* being mainly in the early part of the cotton plant's life, and that of bollworm later; cotton may therefore be protected against either or both, according to whether the insecticide is applied early, late or both early and late. In 1953–54, the three treatments consisted of weekly applications, to run-off, of a spray containing 0.2 per cent. DDT in suspension to 25-ft. lengths of single rows of early-sown (9th June) cotton, separated from each other by seven untreated rows, during August–September, October–November and August–November, respectively; a similarly arranged, untreated row-length was used as control. The treatments were replicated in each of three widely separated areas on the Research Station, and the whole experiment was repeated, with an appropriate alteration in the timing of the treatments, on late-sown (10th August) cotton. Similar experiments were also made in which more restricted periods of protection were employed. Yields were recorded, and crop development was followed throughout the season, on each single-row plot.

On the early-sown cotton, protection from insect attack for the maximum period (four months) did not increase yield, but caused the crop to form earlier. On late-sown cotton, protection by insecticides was accompanied by increases in yield in all localities, although maximum protection did not necessarily give the greatest increase. It thus appears that where a sufficiently long growing season can be achieved by early sowing, as in the elephant-grass zone of Uganda, the cotton crop can replace a considerable loss of flower buds and bolls by subsequent growth, to the extent that total yield is unaffected.

In order to investigate the situation in an area with a single rainy season, shorter growing period, and more intense insect attack, two experiments were conducted at Ilonga, in eastern Tanganyika, where infestations of cotton by *H. armigera* are usually severe. In the first, cotton was sown early (31st January) and completely protected from insect attack by weekly applications of a spray containing 0.2 per cent. toxaphene. Insect damage was then simulated by removing flower buds for periods varying from 4 to 12 weeks. Owing to a high experimental error, yield differences were not statistically significant, but plants that had been disbudded for 12 weeks eventually yielded almost the same amount of cotton, although in a very much shorter period, as those not disbudded at all. The second experiment was a formal comparison of the control of *H. armigera* achieved by different insecticidal treatments on cotton sown late (23rd March). All treatments gave highly significant increases in yield, the best being a dust containing 10 per cent. DDT plus 3 per cent. γ BHC, and an emulsion spray containing 0.8 per cent. DDT + 0.25 per cent. γ BHC, which yielded 968 and 973 lb. seed cotton per acre, respectively, compared with 211 lb. from the control. It thus appeared that even under the conditions at Ilonga, cotton can recover from considerable loss of fruiting bodies, provided it is sown sufficiently early, although late-sown cotton has even less chance of recovery from such damage than it has in Uganda.

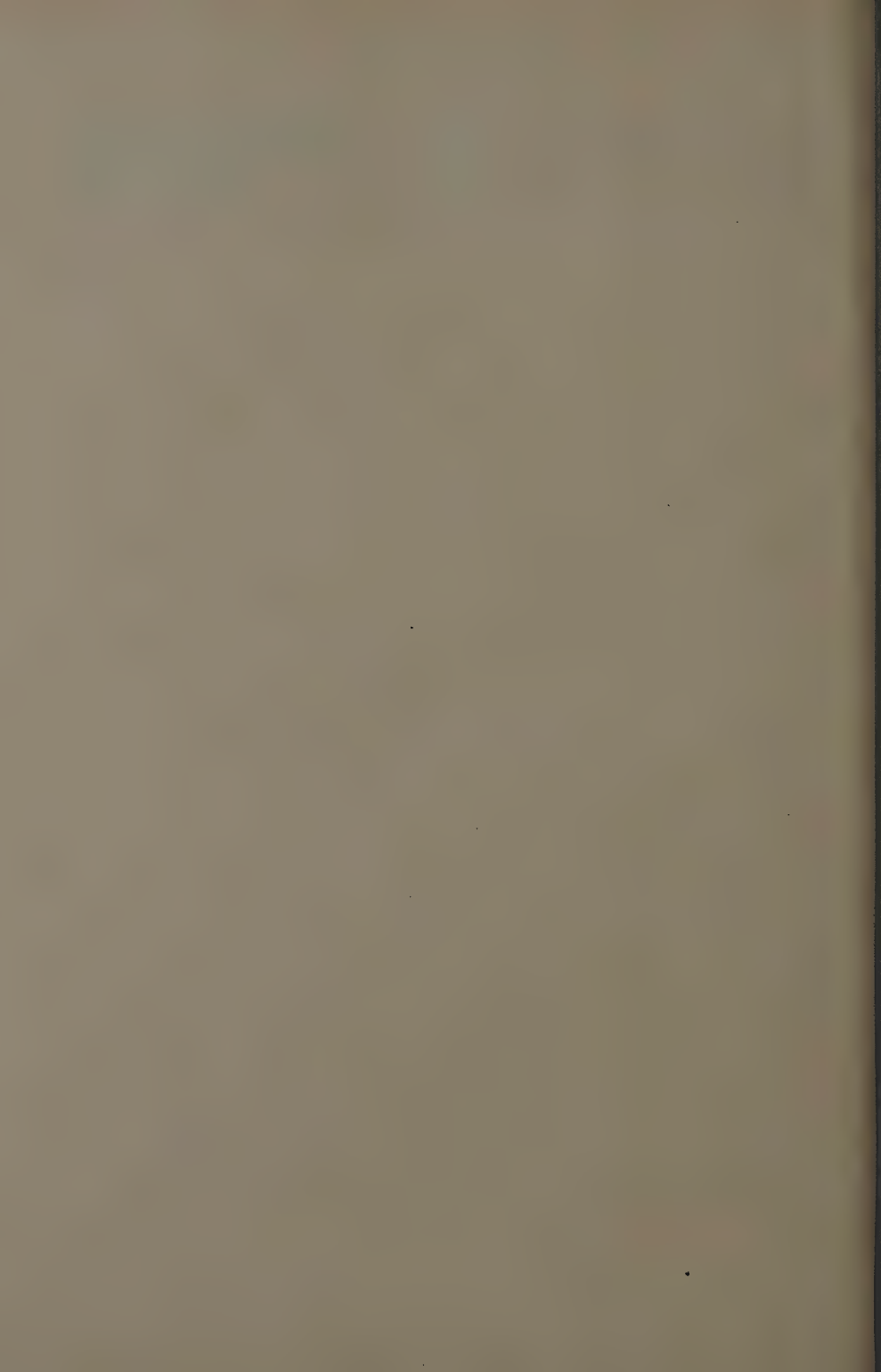
These results, in conjunction with those from similar work elsewhere, including the United States, tend to show that the cotton plant, owing to its method of

growth and crop formation, has remarkable powers of recovery from insect damage. It is only when damage exceeds the point at which recovery is still possible that insecticides will increase yields. Any factors tending to reduce the length of the growing season, or any increase in the severity of the insect attack, will reduce the plant's powers of recovery and make more likely the need for insecticidal control, but it is evident that the presence of insects feeding on the plant does not always mean that such control would be necessary or profitable.

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STUDIES OF CROP LOSS FOLLOWING INSECT ATTACK ON COTTON IN EAST AFRICA.

II.—FURTHER EXPERIMENTS IN UGANDA.

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The study of crop loss following insect attack on cotton in Uganda, already described in Part I of this work (McKinlay & Geering, 1957), was continued for the two further seasons, 1954-55 and 1955-56. The investigation was carried out on the Cotton Research Station, Namulonge, in Buganda Province, and the experimental procedure followed was the same as that of McKinlay & Geering, except that only three periods of protective treatment were used, since it was decided that little additional information would be gathered from those excluded. The three treatments, together with the controls, were replicated eight times in each of three localities, and were applied both to early- and late-sown cotton, as in the 1953-54 experiment. The periods of insecticidal application, and the insect activities that they covered, were as follows:

Coverage	Early sown (mid-June)	Late sown (mid-August)
Bulk of the <i>Lygus</i> attack ...	(a) Aug.-mid-Oct.	(e) Oct.
Bulk of the bollworm attack ...	(b) Mid-Sept.-Jan.	(f) Mid-Oct.-Feb.
Complete season ...	(c) Aug.-Jan.	(g) Oct.-Feb.
Control ...	(d) Unsprayed	(h) Unsprayed

Treatments *b*, *c*, *f* and *g* were continued until boll opening was complete so as to maintain the maximum control against bollworms. This procedure differed from that in the 1953-54 season, when treatment stopped at least two months before picking was completed.

The formulation used in the insecticidal treatment was changed to a spray containing 0.2 per cent. of a mixture of DDT and toxaphene in equal proportions, made up from 50 per cent. paste formulations, and was applied every five days to run-off. Toxaphene was included so as to provide better repellence to *Lygus* than did DDT alone, and the shorter period between applications was considered to give a better residual effect. The increased repellence gained by the inclusion of toxaphene on early-sown cotton is illustrated in fig. 1, by the comparison of the total season's leaf damage for each treatment expressed as a percentage of the control treatment. With the addition of toxaphene in the spray formulation, visible phytotoxic symptoms were shown clearly after the second application of insecticide. A subsidiary experiment showed that these symptoms were not associated with any important reduction in the yield increment (Coaker, 1956).

Leaf Damage.

The results of the experiments considered here have been expressed mainly in terms of the total crop formed and its time of harvesting, but extensive use has also been made of leaf damage caused by *Lygus* as an index of its activity. It will be convenient, before dealing with the yield results, to discuss leaf-damage estimates during the three seasons in which these experiments have been made.

A detailed description of the damage caused to cotton by *Lygus vosseleri* Popp. was given first by Hancock (1935) and later by Taylor (1945). The characteristic feature of *Lygus* damage is the holing and tattering of the leaves resulting from the adults and nymphs feeding on them when they are young and folded. The insertion of the proboscis and injection of saliva kills the surrounding tissue, the dead area showing as a small black scar as the leaves

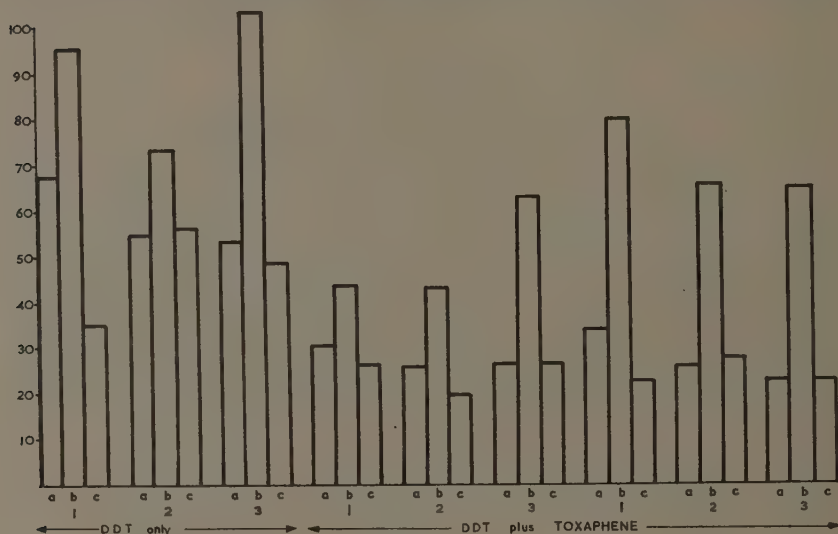


Fig. 1.—Comparison of *Lygus* leaf damage under conditions of three insecticidal treatments in each of three localities during three seasons, 1953-56. Histograms show values of leaf-damage index, totalled over three seasons, expressed as percentages of the values on the control plots, for each locality. 1-3, localities (1, Sendusu; 2, Nalumu; 3, Kirimantungo); a-c, periods in which weekly applications of insecticide were made (see text, p. 851).

begin to unfold. As they expand, severely damaged leaves become tattered, while leaves that have only been lightly attacked develop a few holes. The degree of final tattering is probably misleading in its relationship to the initial damage inflicted on the young, folded leaves. One insertion of the stylets may pass through more than one point of the leaf surface, effecting severe tattering with unfolding and expansion.

For many years entomologists in Uganda have made use of the characteristic damage to the leaf as an index of the severity of *Lygus* attack. Gwynn (1936) described an arbitrary scale on which leaf damage was then recorded. The method used was to take the four fully unfolded, uppermost leaves of the main stem and the first leaf on the sympodial branch subtended by the fourth leaf. Marks were allotted for each leaf on the following scale:

- 0 = No damage.
- $\frac{1}{2}$ = Slight damage.
- 1 = Slight to moderate damage.
- 2 = Severe damage.

The total score for the five leaves was the index for each plant and gave a sample of damage done during the period between successive observations (that is,

every two weeks). The main criticism of this technique is that, in the absence of any standard of comparison, the assessment of the degree of damage was a subjective one. Subsequently, the method was modified to include observations on the top five opened leaves on the main stem, which were graded on an arbitrary scale, from zero to ten. Details of this method, which is subject to the same criticism, have not been published.

The method which was used at Namulonge by the author's immediate predecessors (the following description being extracted from notes left by Q. A. Geering), was essentially similar to Gwynn's original method and was as follows:—

The four topmost main-stem leaves that were fully expanded were graded. Counting the topmost leaf that had begun to unfold as number one, this meant grading leaves three, four, five and six, counting downwards. Observations were made every two weeks and gave a cumulative index of the damage done to each of these leaves as they passed in succession through the young stage in the

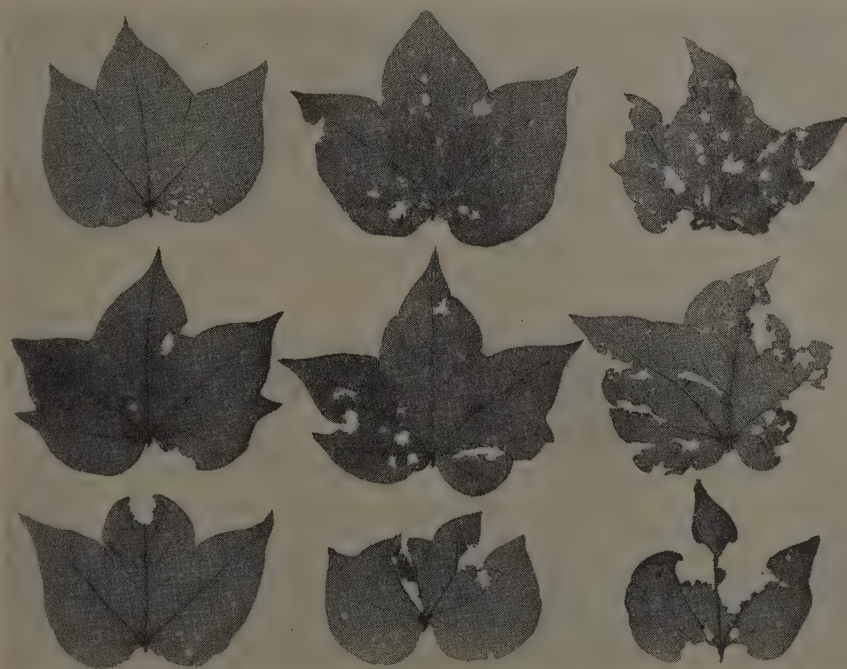


Fig. 2.—Standard grades of damage to cotton leaves caused by *Lygus* spp. The columns represent (from left to right) grades A, B and C; the rows illustrate the range of types of perforation that can develop. For explanation of method of use, see text (p. 854).

growing point of the main stem. It is essential to grade only fully expanded leaves, for not until this stage are maximum tearing and shredding apparent. Moreover, at this stage the leaves are of comparable size.

In order to reduce the subjective element to a minimum, a set of leaves representative of the range of typical damage, from slight to severe, was prepared and photographed (fig. 2). Three examples of the type of perforation that

can develop are given in each category illustrated. A full-plate reproduction was mounted on a sheet of white cardboard, $8\frac{1}{2}$ in. \times 13 in., covered with a transparent plastic sheet and used in the collection of all leaf-grade data. The expanse of white below the photograph was placed behind the leaf being examined, as a background, so that the holing could be seen more clearly and compared immediately with the standard grades. A leaf with no damage is scored zero. Leaves showing damage equal to or less than A are scored one; those between A and B or equal to B are scored two; those between B and C or equal to C are scored 3 and any showing worse damage than C are scored 4. During the first year's crop-loss experiment (1953-54), five plants in each plot of 25 plants were sampled by the above method, once every two weeks.

The main criticism of the methods described is the unnecessary repetition of leaf sampling. It has been shown that under local conditions one new main-stem node is added approximately every ten days during the normal growing period of the plant. Inevitably some leaves were sampled more than once and therefore the index did not accurately reflect *Lygus* activity during the previous two weeks. During the 1954-55 season a comparison was made of the four-leaf method and one in which only the two uppermost fully open leaves were sampled (numbers three and four already described). The two methods yielded similar mean maximum and mean minimum values for the leaf-damage index, but the two-leaf method showed a greater sensitivity to *Lygus* fluctuations, the values for the leaf grade falling more rapidly following insecticidal applications. The two-leaf method has the further advantage that a larger sample of plants can be examined in a given time. During the experiment in the two seasons under discussion all 25 plants in each plot were sampled, that is, 200 plants per treatment every two weeks.

Lygus damage indicated by leaf tattering does not usually occur uniformly

TABLE I.

Comparison of the variability obtained with the four- and two-leaf methods of estimating the *Lygus* leaf-damage index.

Locality	Treatment	1953-54		1954-55				1955-56	
		4-leaf		4-leaf		2-leaf		2-leaf	
		Mean	C.V.	Mean	C.V.	Mean	C.V.	Mean	C.V.
Sendusu	a	1.3	56.5	0.6	45.3	0.5	29.2	0.9	36.6
	b	1.3	19.6	0.7	56.3	0.8	20.5	1.9	14.6
	c	0.6	52.6	0.5	45.8	0.5	19.3	0.8	29.3
	d	1.5	12.3	1.8	24.8	1.8	12.9	2.5	18.5
	(control)								
Nalumu	a	0.9	11.0	0.4	50.7	0.5	75.6	0.8	23.5
	b	1.2	45.4	0.7	31.7	0.7	30.1	0.4	21.9
	c	0.9	11.8	0.3	48.7	0.4	44.9	0.4	37.3
	d	1.6	24.1	1.8	30.6	1.7	47.9	1.1	41.2
	(control)								
Kirimantungo ..	a	0.8	31.9	0.6	29.8	0.5	42.2	0.4	36.7
	b	1.5	12.2	1.1	40.9	1.1	39.5	1.2	25.1
	c	0.8	20.5	0.6	41.4	0.5	43.9	0.4	31.4
	d	1.8	17.3	2.1	21.3	2.0	13.9	1.8	15.5
	(control)								

C.V. = coefficient of variation (expressed as percentage).

Mean = Seasonal mean of leaf-damage index.

and is often more serious on the larger and more vigorous plants. Manning (1950) has shown that a close relationship exists between plant height and *Lygus* damage, which may be due to an earlier attraction to vigorous plants. Unless, therefore, a large sample of plants is taken, the results will show much variability. The standard errors of means calculated for both methods show somewhat less variability in the results obtained using the two-leaf method. Data are given in Table I.

In the past the leaf index was used only as a measure of *Lygus* activity and its relation to any reduction of fruitfulness was unknown. However, before considering this aspect of the problem it is important to know whether the measure of leaf damage is in any way a reliable index of the population of *Lygus* present. The reason for recording leaf damage lies in its possible use as an indirect and simple measure of the effect on the cotton crop of any given population of *Lygus*; as such it would be valuable in surveying the extent of *Lygus* damage over wide areas, and as a fairly rapid and reliable indication of yield loss. To date it has been useful in measuring the degree of control of *Lygus* achieved by insecticide treatments. It was not until the single-row technique was devised by McKinlay (1953) that valid estimates could be obtained of the effect of protecting cotton plants from *Lygus* damage. Using this method, it proved possible, by suitable treatments, giving different periods of protection, to obtain plants representing a range of leaf-damage indices, and also, by sweeping, samples of the *Lygus* populations associated with these indices. Such information was obtained from an experiment carried out, by courtesy of the Senior Entomologist of the Department of Agriculture, at Serere Experiment Station in the Eastern Province of Uganda and is summarised in Table II. The

TABLE II.

Relationship between total numbers of *Lygus* and associated index of leaf damage observed on plots representing 10 insecticide treatments.

Total <i>Lygus</i> caught	..	49	64	69	77	84	86	89	118	127	133
Index of leaf damage (totals for season)	..	592	773	750	1025	924	1095	1138	1335	1372	1225

$$R^2 = +0.851 \ (P < .001).$$

(Experiment conducted at Serere in the Eastern Province, Uganda.)

Lygus populations are represented as the totals of adults and nymphs caught on 17 occasions at weekly intervals throughout the period of *Lygus* activity. On each occasion a single sweep was made on each cotton plant on eight replicates of 30 feet of row on each of the 10 treatments. Leaf damage was estimated on nine occasions at fortnightly intervals covering the same period; and for each treatment and each occasion eight replicates of five plants were graded by the four-leaf method, i.e., 160 leaves per occasion. The correlation between these two quantities is highly significant. Data are not available for the relation between *Lygus* population and leaf-damage index on cotton of the BP52 variety at Namulonge, but there seems no reason to doubt that a similar correlation would be found.

Figures were also collected at Namulonge for the relation between leaf-damage index and the shedding of fruiting bodies* consequent on damage by Mirids. The effect of insecticidal treatments was to reduce damage to both leaves and fruiting bodies, but within each of the four treatments tested at three localities in the two seasons, 1954-55 and 1955-56 (24 comparisons in all), there

* The term "fruiting body" is used to cover both flower buds and bolls. The term "fruiting point" denotes the node at which a fruiting body is formed.

was in no case a significant association between leaf-damage index and loss of fruiting bodies from Mirid attack.

No statistically significant relationship was obtained between leaf damage and yield. Since yield is the measure of ultimate loss incurred by the plant, leaf damage cannot be used as an indication of crop loss under the conditions and at the levels of *Lygus* infestation experienced locally. Plants can be found that have suffered severe leaf damage and also considerable loss of fruiting bodies, but at Namulonge this is exceptional. It may well be that with less favourable climatic conditions and larger *Lygus* populations, the reduction of leaf area caused by *Lygus* feeding would itself produce a strain on the plant sufficient to cause loss of fruiting bodies. Goodman (1956) has recently shown in the Sudan that in plants with one or more than one leaf in eight removed the yield is diminished. Conditions in the part of Uganda of which Namulonge is representative are such that a large measure of recovery is possible from losses of both leaf area and fruiting bodies suffered in the early part of the season. As it is early in the season that most of the direct losses from *Lygus* occur it is to be expected that the crop will recover from *Lygus* attack in time to allow a full crop to be produced, and no relationship between leaf damage and crop size will therefore be apparent.

Shedding of Fruiting Bodies.

Before considering the results obtained it should be recalled that the cotton plant naturally sheds a considerable number of fruiting bodies apart from losses attributable to the attacks of pests and diseases. Shedding of fruiting bodies caused by insect damage may affect the timing of the crop, but unless it is in excess of natural shedding it is unlikely to alter crop size, since otherwise it merely alters the distribution on the plant of the shedding that would have taken place in any case.

Counts of total fruiting points were not made in the two seasons under discussion. It was desired to determine the direct cause of fruiting-body loss with respect to the proportional degree of damage caused by the two groups of pests within the crop, namely, *Lygus* spp. and bollworms. The procedure adopted was that all buds and bolls that had been shed, or were about to be shed,† were collected every three days throughout the season from all 25 plants in each plot, i.e., from 200 plants per treatment per locality on each occasion. All those collected were sorted in the laboratory.

It was assumed that any visible insect damage was a primary cause of shedding. Shedding caused by Heteroptera in the crop was characterised by a puncture mark on the calyx cup or the petals, in the case of flower buds, and by a puncture mark on the external surface of the carpel, or by proliferating cell tissue on its inner surface, in the case of bolls. No attempt was made to classify the damage according to the species of Heteroptera causing it, all shed buds or bolls so affected being classed together as "bug-punctured". Buds and bolls shed after damage by bollworms, of which *Heliothis armigera* (Hb.) is the most important (Coaker, 1955), were similarly grouped together. Such damage was identified by a small circular hole, upwards of 1 mm. diameter, at the base of the bud or in the boll wall. Fruiting bodies that showed neither bug, bollworm nor disease damage were classified as shed due to "other causes", probably physiological in nature. If the fruiting body was damaged by both types of insect, it was classed under the type of damage that appeared the oldest and most severe. The number of buds and bolls damaged by primary disease infections was always small, and it was therefore possible to omit them from the discussion of this experiment.

† Buds in which the abscission layer is well developed can be detected by the characteristic flaring of the bracteoles.

TABLE III.

Causes of shedding on cotton treated with insecticides for various periods.

Season	Treatment	Cotton sown early (mid-June)					Cotton sown late (mid-August)				
		% Shedding			S.E. for differences between totals	Total fruiting bodies shed	Treatment	% Shedding			S.E. for differences between totals
		Boll-worms	Bugs	Other causes				Boll-worms	Bugs	Other causes	
1954-55	a	1.8	15.9	79.2	± 51	2327	e f g h (control)	3.5	18.3	78.1	± 63
	b	0.6	14.8	83.0		1919		0.4	7.4	92.2	
	c	0.9	17.5	78.2		2122		1.7	8.7	89.4	
	d (control)	10.8	27.2	59.5		2229		18.0	20.8	61.1	
P (for d against a, b, c)		0.001	0.001	0.01		N.S.		0.001	0.01	N.S.	
1955-56	a	0.5	4.5	94.4	± 53	1927	e f g h (control)	2.4	9.3	88.2	± 57
	b	1.1	3.1	95.7		1655		3.8	0.2	96.0	
	c	0.2	1.7	97.9		2081		0.9	0.1	98.9	
	d (control)	16.8	24.1	58.8		1766		9.0	27.1	63.9	
P (for d against a, b, c)		0.001	0.001	0.001		N.S.		0.001	0.001	0.05	

The results show the proportion of the shedding attributable to the various causes, the figures against each treatment representing the numbers of shed fruiting bodies in each category expressed as percentages of the total. The figures for shedding caused direct by disease are omitted. All data from Nalumu. For explanation of classification of shedding see p. 856. For explanation of treatments (a-h), see text.

From the results obtained (Table III) it can be seen that buds and bolls shed from "other causes" constituted the majority of those examined. Shed bolls predominated. In most localities, the total amount of shedding was roughly the same in all treatments, that due to "other causes" tending to balance that due to insects, so that if the latter was high the former was low, and *vice versa*.

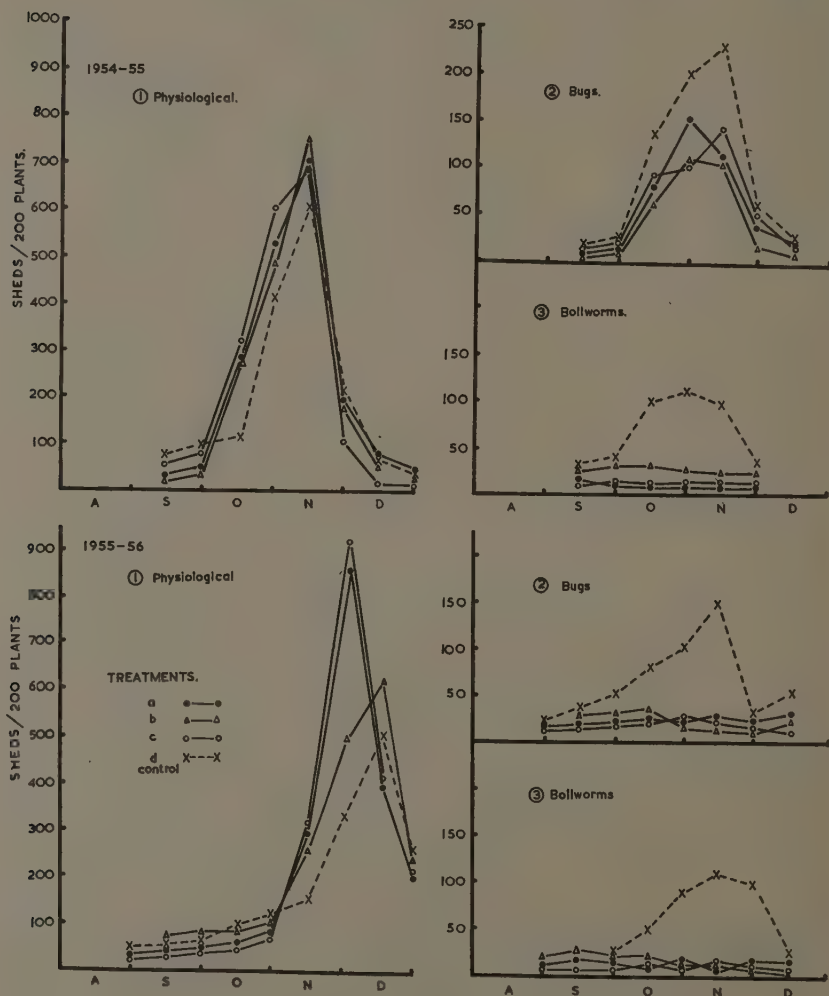


Fig. 3.—Numbers of fruiting bodies shed from various causes on cotton sown early (mid-June) at one locality (Nalumuli) in successive seasons. Data plotted as 2-weekly totals from 200 plants. *a-d*, periods in which weekly applications of insecticide were made (*a*, Aug. to mid-Oct.; *b*, mid-Sept. to Jan.; *c*, Aug. to Jan.; *d*, untreated control); A, S, O, N, D, successive months, August–December.

Examination of the rate of shedding in the early-sown cotton (fig. 3) shows that chemical protection of the fruiting bodies early in the season causes most of the shedding not due to insects to occur early in the season; conversely on the untreated plots, and also those treated with insecticides late in the season, such shedding occurs later. This evidence is in line with the results obtained by McKinlay & Geering (1957), and many other workers, demonstrating an increased rate of shedding coincident with the development of the crop of bolls. It is evident that some overriding factor must have been controlling the balance of yield. Since in all of the treatments, shedding due to insects was exceeded by that due to other causes, even in the control treatment, it is suggested that insect-caused shedding can be disregarded as a cause of loss of final yield.

It is interesting to note that most of the shedding, whether caused by insects or physiological conditions, occurs within a definite period in each season: in 1954-55, between October and November, and in 1955-56, between November and December (fig. 3). These periods coincide with those demonstrated by Farbrother (1954, 1955) as being of low relative turgidity in the cotton plant, indicating a strain on the plant due to insufficient water availability in relation to the size of crop then maturing. Since the peak insect populations are not restricted to these periods alone, the results suggest a close association of shedding or damaged fruiting bodies with periods of stress within the plant.

The total numbers of shed buds and bolls collected by the method described differed very considerably from those recorded by Farbrother (1954, 1955), which were derived from periodical counts of all the fruiting points on the plant. Only some 20 per cent. of all the fruiting bodies lost by the plant were recovered. The difference is due to the impossibility of collecting the buds that are shed when they are very small, which Farbrother (1953, see also McKinlay & Geering, 1957, p. 841), who has termed them "match-head" buds, has shown to be an important proportion of all those shed. It is assumed that since the very small buds shed cannot be directly attributed to physical damage by insects, the proportions attributed to the two groups of insects, amongst the total fruiting bodies collected, should indicate satisfactorily their respective contributions to the damage caused to the crop at the fruiting stage of development.

There was little between the two types of pests in the amount of shedding caused on the control plots, but the insecticidal treatment of the bollworms was the more effective. The rapid movements of adults and the slow action of the insecticides used in repelling them probably account for the failure to achieve full control in the case of *Lygus*. The amount of shedding that could be ascribed to bugs or to bollworms was significantly reduced by insecticidal treatment. Conversely, the amount of non-insect shedding was greater in the plots treated with insecticides, significantly so in nine out of the 12 comparisons.

To sum up, it is suggested that a natural balance occurred between shedding due to insects and to physiological causes, so that under the conditions at Namulonge the total shedding tends to be similar, irrespective of the nature or timing of the insecticidal treatments. The total amount of shedding was thus determined by some factor other than insect attack, and, so long as the latter was of moderate proportions, the overall fruiting of the plant was not affected by the shedding caused by insects.

Time of harvesting and yield.

The data presented above suggest that, under the conditions experienced, insect damage that results in shedding, does not have a serious effect on the final yield. However, damage caused to bolls that have set (*i.e.*, that have passed the stage in development when physical damage would cause them to shed), must inevitably produce a direct loss in final yield. The range of damage

to the boll caused by insect feeding extends from partial damage of one locule to the complete destruction of all the boll contents. Insect damage alone does not usually result in complete destruction. Where complete loss occurred it was invariably associated with invasion by fungi or bacteria through the lesion caused by the insect on the carpel. Both *Lygus* spp. (and various other Heteroptera) and bollworms are capable of causing the full range of damage described.

TABLE IV.

Quality of the seed cotton harvested from plots treated with insecticides for various periods.
Percentages of loculi in each of three grades of quality.

Season	Early sown (mid-June)				Late sown (mid-August)			
	Treatment	Grade			Treatment	Grade		
		A	B	C		A	B	C
1954-55	<i>a</i>	92.4	4.8	2.8	<i>e</i>	72.7	19.7	7.6
	<i>b</i>	95.9	2.3	1.8	<i>f</i>	77.0	15.4	7.6
	<i>c</i>	94.4	3.3	2.3	<i>g</i>	79.0	13.5	7.3
	<i>d</i>	80.6	14.6	4.8	<i>h</i>	66.8	20.9	12.3
	(control)				(control)			
1955-56	<i>a</i>	95.6	3.2	1.4	<i>e</i>	79.6	13.3	7.1
	<i>b</i>	97.2	1.9	0.9	<i>f</i>	95.6	2.8	1.5
	<i>c</i>	97.2	1.8	1.0	<i>g</i>	94.9	3.5	1.6
	<i>d</i>	92.1	4.9	3.0	<i>h</i>	80.6	12.3	7.1
	(control)				(control)			

Grades of damage: A, clean; B, partially damaged; C, completely damaged.

All data from Nalumuli. For explanation of treatments (*a-h*), see text.

To determine the extent to which the two main groups of insects contributed towards the final loss, all ripe, split bolls were collected from each plot every week and examined in the laboratory, where they were separated into good, partially damaged and completely damaged locules. The data for this analysis from one locality are given in Table IV. Locules directly damaged by disease organisms were not considered. Those damaged by insects were classified according to the type of damage, which is best indicated on the boll wall, through which all insect feeding must pass. Similar symptoms, typical of those described on shed buds and bolls, are found on the dried boll wall of the split damaged bolls.

Only in one of the seasons (1954-55) did the average proportion of locules damaged in the early-sown cotton approach 20 per cent., including some five per cent. completely damaged. In the late-sown cotton, the damage was more serious, averaging one-third of the locules in 1954-55, including over 12 per cent. completely damaged.

The material was further classified as bug-damaged or bollworm-damaged. Data from one locality are presented in Table V. At least two-thirds of the damage on the untreated controls in the early-sown cotton was caused by bollworm, but the insecticidal treatments used proved most effective against this class of pest, as is evident from the fact that the figure for bollworm damage is in most cases less than the corresponding one for bug damage.

Curves showing cumulative weekly pickings from samples in one locality for the early-sown crops in 1954-55 and 1955-56 are given in fig. 4. They illustrate the influence on the development of the crop of the different periods of insecticidal application. The insecticidal treatment (*b*) applied at the end of the season

TABLE V.
Relative damage due to bollworms and to various Heteroptera.

Season	Treatment	Early sown (mid-June)				Treatment	Late sown (mid-August)			
		Grade B		Grade C			Grade B		Grade C	
		B.W.	B.P.	B.W.	B.P.		B.W.	B.P.	B.W.	B.P.
1954-55	<i>a</i>	37.4	30.6	59.8	16.4	<i>e</i>	32.2	57.6	43.1	47.5
	<i>b</i>	25.6	44.7	31.5	4.4	<i>f</i>	8.5	71.5	13.6	59.5
	<i>c</i>	33.8	30.4	39.7	18.7	<i>g</i>	5.1	89.4	7.1	82.8
	<i>d</i>	69.9	20.7	65.1	21.2	<i>h</i>	31.1	59.7	40.2	50.8
	(control)					(control)				
1955-56	<i>a</i>	31.5	63.4	48.3	51.7	<i>e</i>	54.6	44.1	69.2	29.9
	<i>b</i>	23.9	71.7	27.7	66.3	<i>f</i>	25.5	67.2	21.7	73.9
	<i>c</i>	10.4	72.4	15.8	74.6	<i>g</i>	19.9	77.2	29.2	63.4
	<i>d</i>	73.4	22.8	86.8	10.5	<i>h</i>	50.4	48.8	64.8	34.7
	(control)					(control)				

The figures show the number of loculi in which the damage is attributable to bollworms (B.W.) and to various Heteroptera (B.P.), expressed as a percentage of the total loculi in the given grade and treatment. Grades of damage: B, partially damaged; C, completely damaged. For explanation of treatments (*a-h*), see text. All data from Nalumuli.

(mid-September to January) was on the whole the best, in terms of final yield (see Table VI). In considering the season as a whole this is not surprising, since early loss of fruiting bodies due to insect attack stimulates further growth and in due course a later crop, which in turn is protected from insects during the period of the most damaging attack. Protection throughout the season (treatment *c*)

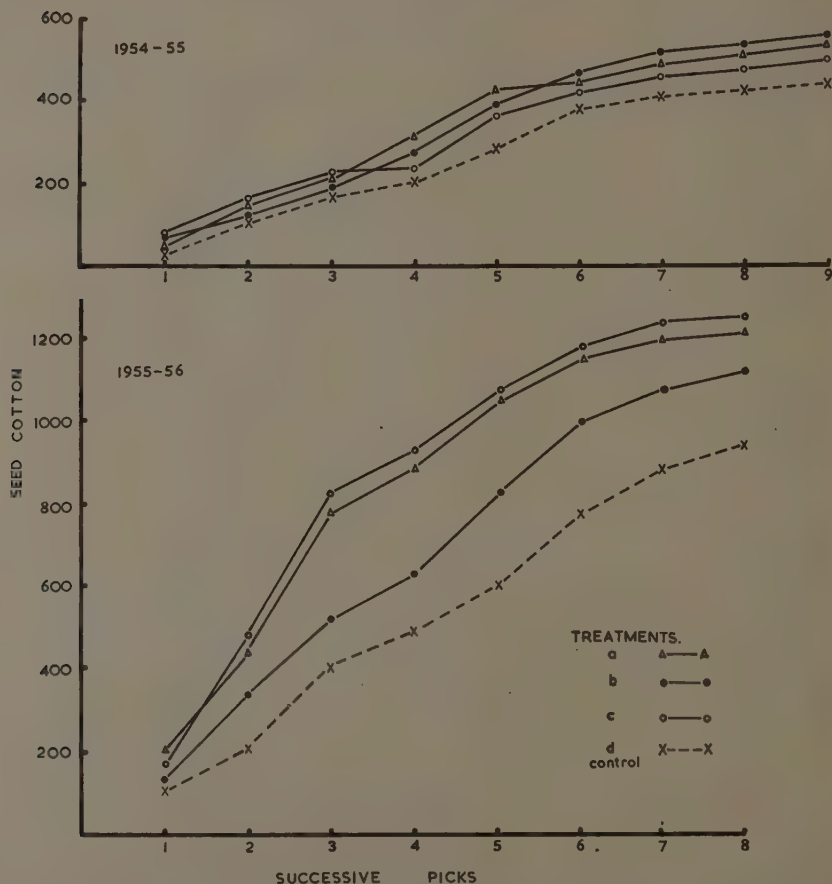


Fig. 4.—Cumulative picking curves in successive seasons at Nalumuli, expressed as lb. seed cotton per acre. *a-d*, periods in which weekly applications of insecticide were made (*a*, Aug. to mid-Oct.; *b*, mid-Sept. to Jan.; *c*, Aug. to Jan.; *d*, untreated control).

prevents the early loss of fruiting bodies, and also, therefore, the consequent formation of a larger plant structure; the final yield thus tends to be lower than in the case of late treatment. Treatment (*b*) and the controls (*d*) tended to make their crop later in the season than did treatments involving early insecticidal applications. In a similar curve for the 1953-54 season (McKinlay & Geering,

1957), this characteristic is more clearly defined owing to the extended harvesting period of that year.

The unfavourable weather in 1954-55 did not entirely prevent recovery from early shedding losses, as might have been expected from the low water availability at the time when it was most required (Manning & Farbrother, 1955). The

TABLE VI.

Total yields of seed cotton (expressed in lb. per acre) and percentage clean (AR) from cotton treated with insecticides for various periods.

Season and locality	Early sown (mid-June)			Late sown (mid-Aug.)		
	Treatment	% AR	Yield	Treatment	% AR	Yield
1954-55 Sendusu	<i>a</i>	96.9	591	<i>e</i>	72.1	189
	<i>b</i>	97.9	528	<i>f</i>	92.9	305
	<i>c</i>	97.1	516	<i>g</i>	92.7	316
	<i>d</i>	90.8	598	<i>h</i>	70.3	211
	(control)			(control)		
	S.E. (diff.) ± 114			S.E. (diff.) ± 109		
Nalumuli ..	<i>a</i>	96.4	494	<i>e</i>	85.9	182
	<i>b</i>	98.2	513	<i>f</i>	90.4	160
	<i>c</i>	97.4	477	<i>g</i>	90.9	167
	<i>d</i>	90.5	476	<i>h</i>	81.5	162
	(control)			(control)		
	S.E. (diff.) ± 98			S.E. (diff.) ± 73		
Kirimantungo ..	<i>a</i>	94.5	437	<i>e</i>	80.5	196
	<i>b</i>	94.8	537	<i>f</i>	94.8	174
	<i>c</i>	94.2	432	<i>g</i>	90.8	211
	<i>d</i>	85.7	339	<i>h</i>	77.9	138
	(control)			(control)		
	S.E. (diff.) ± 97			S.E. (diff.) ± 87		
1955-56 Sendusu	<i>a</i>	95.4	829	<i>e</i>	87.1	469
	<i>b</i>	95.2	1129	<i>f</i>	97.4	619
	<i>c</i>	96.4	961	<i>g</i>	97.1	608
	<i>d</i>	87.9	822	<i>h</i>	82.6	389
	(control)			(control)		
	S.E. (diff.) ± 229			S.E. (diff.) ± 132		
Nalumuli ..	<i>a</i>	98.4	1201	<i>e</i>	89.3	438
	<i>b</i>	98.7	1091	<i>f</i>	98.4	508
	<i>c</i>	98.5	1218	<i>g</i>	97.9	499
	<i>d</i>	96.9	973	<i>h</i>	90.7	466
	(control)			(control)		
	S.E. (diff.) ± 215			S.E. (diff.) ± 120		
Kirimantungo ...	<i>a</i>	98.6	1178	<i>e</i>	91.2	201
	<i>b</i>	99.0	1268	<i>f</i>	97.5	361
	<i>c</i>	99.0	1205	<i>g</i>	97.6	401
	<i>d</i>	99.6	1195	<i>h</i>	90.5	343
	(control)			(control)		
	S.E. (diff.) ± 154			S.E. (diff.) ± 87		

For explanation of treatments (*a-h*), see text.

final yields (Table VI) of the different treatments both on early- and late-sown cotton during both seasons did not differ significantly.

To sum up, it appears from the results obtained that damage caused to bolls that have set, in particular by bollworms, is the most important loss inflicted on the crop. However, that loss together with loss inflicted earlier in the season is not sufficiently large to justify further investigation of control measures.

Discussion and Conclusions.

It was originally intended that an experiment to determine the status of insect pests in the cotton crop at the Cotton Research Station, Namulonge, should be continued for a number of years, so that the effect of variation between the seasons could be studied. However, the three seasons during which the experiment was conducted have proved sufficiently diverse to be regarded as covering the range of conditions likely to be experienced, and since they have all yielded basically similar results, an early conclusion may be justified.

During the first season (1953-54), favourable weather conditions enabled the plants to recover from early shedding losses caused by insect attack (McKinlay & Geering, 1957). It was believed that such recovery could only occur under optimum environmental conditions, namely, a long growing season favoured by well distributed rainfall. In the second season of the experiment (1954-55), less favourable conditions prevailed, reducing yields by half, but even so, recovery from early shedding loss was similar to that of the previous season. The third season (1955-56), which could be considered as average, also produced similar results, with yields approximating to those of the first season, in both the treated and untreated cotton. Shedding caused by insects was greatest during 1954-55, but when the three localities in which the experiment was replicated were considered as a whole, worthwhile increases following insecticidal treatment were not obtained.

It is suggested that the main differences in final yield between the various treatments were brought about by the late attack of bollworms on the bolls that were already set, and that, therefore, if any future insecticidal control were to prove worthwhile, it would be a treatment applied over the latter part of the season.

The points under discussion have for the most part referred to early-sown cotton. Late-sown cotton responds more consistently to insect control. This is to be expected, since crops growing late in the season do not have time to recover from pest losses.*

The final yields of the late-sown cotton were always considerably lower than that planted two months earlier, and for this reason alone little significance has been attached to this part of the experiment in determining the pest status. Yields from late-sown cotton, even when this was protected by insecticides from pest losses, were never so large as those obtained from early sowings that did not have insecticidal protection. It is, in fact, far better to ensure a big crop by early sowing than to rescue with insecticides the meagre produce of a late-sown crop.

The following conclusions can be drawn from the three seasons' work. It must be emphasised that they refer to the conditions characterised by the Cotton Research Station, Uganda.

(1) *Lygus* spp. and other Heteroptera are not responsible for any serious reduction in the final yield of early-sown cotton. The length of the season makes possible recovery from early fruiting-point loss. The effectiveness of this

* It should also be mentioned here that the late-sown cotton was always grown adjacent to early-sown cotton and may have been influenced by insect migration from the latter. This, however, was never demonstrated by leaf-damage indices.

recovery is controlled by weather conditions. In late-sown cotton, however, loss of the earlier-formed flower buds cannot usually be compensated for by further growth and fruiting-point production, and consequently *Lygus* attack will reduce the crop.

(2) Control of *Lygus* by insecticides results in the retention of early-formed fruiting bodies and thus in an earlier crop. The production of an earlier crop, harvested over a shorter period, is in itself of value, but only in a highly developed system of intensive agriculture would the advantage conferred be sufficient to justify the use of insecticides.

(3) Bollworm damage can be inflicted both on the bud and young boll stages causing shedding, usually to a lesser extent than that caused by *Lygus*, and on the larger, set bolls. In early-sown cotton, buds and young bolls that have shed may be replaced, but damage to large bolls constitutes a direct loss. However, the losses have not yet been large enough to justify further investigation of the economics of control measures.

Summary.

The study of crop loss following insect attack on cotton in Uganda, described in Part I of this work, was continued for two further seasons (1954-56), using the same experimental procedure, except that half the DDT in the spray formulation was replaced by toxaphene, only three periods of protective treatment were used for each date of sowing, and the late-season ones were continued until boll-opening was complete, so as to give maximum control of bollworm.

Methods are described of estimating an index of leaf damage caused by *Lygus* spp., based on comparisons with standard grades. At Serere (Eastern Province), the index of leaf damage was closely related to *Lygus* populations in the crop, and at Namulonge (Buganda) it was markedly reduced on plots protected from *Lygus* attack, but in the 1954-56 experiments no relationship could be found between the mean seasonal index and the loss of fruiting bodies attributable to *Lygus* attack, or the final yield.

Analysis of the cause and incidence of shedding of developing fruit bodies (flower buds or young bolls, the latter predominating among those recovered) showed that the total amount of shedding was roughly the same whatever the treatment, that due to insect damage tending to be balanced by that due to other causes, which were probably physiological in nature. There was thus some over-riding factor, other than pest attack, that governed the crop finally held by the plant.

Damage caused by insects to older bolls, which do not shed, constitutes a real loss that is reflected in the final harvest. The proportion of clean seed cotton was higher in the treated plots, particularly in the early-sown cotton, in which analysis of the harvested locules showed that the damage was predominantly due to bollworm, but there were no significant differences in yield between the various treatments in either date of sowing or in either season.

It is concluded that under such conditions as those experienced at the Research Station in the three seasons of the experiments, insect damage to cotton is small in relation to other environmental factors governing crop production.

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